Microhabitat requirements of the great crested newt (*Triturus cristatus*) in a woodland area

Thesis

Microhabitat preferences of the great crested newt *Triturus cristatus* in a woodland area

Laurence Edward Jarvis

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Department of Biological Sciences

Faculty of Science

Open University

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Abstract

The great crested newt *Triturus cristatus* is one of Europe's fastest declining amphibian species. This thesis aimed to increase our knowledge of the microhabitat use and ecology of great crested newts within a woodland area in the British Isles and suggest ways of improving measures for conservation. The study was conducted at Epping Forest Field Centre and surrounding deciduous woodland at High Beach, Epping Forest, which is situated to the north-east of London. Capture-mark-recapture of adults in four ponds over 5 years within a 1 km area revealed that great crested newts exhibited large fluctuations in detection probabilities, highlighting the importance of calculating detection before making inferences of population size or survival. Apparent annual survival was constant between ponds and years; male survival was 0.40 compared to 0.58 in females. Only 11 inter-pond movements were recorded. Many terrestrial juveniles occupied cover objects close to breeding ponds for many months of each year. Growth rates were fastest in smallest individuals (35 to 39 mm SVL) and juvenile body condition varied through each year. Juveniles had high monthly survival (0.56 to 0.77) and a low constant apparent annual survival of 0.19. Adult males exhibited lowest body condition index (BCI) in March and June while females had high BCI in March and lowest in April. Larger males with a higher body condition were more likely to possess taller crests. Experiments showed increased mortality in great crested newt embryos when raised in the presence of caged predatory sticklebacks compared to controls. Great crested newt larvae utilised vegetated microhabitats for a significantly greater proportion of the time when in the presence of caged predatory sticklebacks but not when faced with non-predatory crucian carp. Finally, great crested newt females exhibited a non-random distribution in egg laying. Individuals preferred to lay eggs on clean compared to occupied strips. These findings are discussed in relation to improving our understanding of great crested newt ecology and conservation.

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Chapter 1. Introduction and background

1.1. Amphibian declines

1.1.1. Global perspectives

Amphibians worldwide are experiencing dramatic declines (Gardner et al., 2007; Houlahan et al., 2000; Lips et al., 2004; Pounds et al., 2006) with over one third of species being threatened with extinction (Stuart et al., 2004). Currently 1,910 of the planet's 6,312 amphibians are in danger of extinction, making them the most threatened group of species known to date (IUCN, 2011). Species most at risk are those within Neotropical regions at higher altitudes, occupying streamside habitats (Stuart et al., 2004). Causes of declines appear to be complex and in many cases result from synergistic interactions between many factors (D'Amen & Bombi, 2009; Pounds et al., 2006). Habitat loss and fragmentation have had the greatest impact on amphibian declines worldwide (Becker & Loyola, 2008; La Marca et al., 2005; Nyström et al., 2007; Lips, 1998). The Global Amphibian Assessment (GAA) identified habitat loss as affecting 89% of all threatened species in the New World, which is three times greater than any other threat (Gardner et al., 2007). In aquatic breeding species losses of breeding sites have had detrimental effects (Curando et al., 2011; Swan & Oldham, 1993). Destruction of terrestrial habitats, altering of the water table, development and isolation caused by construction of roads have also had negative impacts on many populations (Becker et al., 2009; Beebee, 1973). Another major factor implicated in amphibian declines worldwide has been the emergence of infectious diseases, notably *Batrachochytrium dendrobatidis*,

the causative agent of the fungal infection chytridiomycosis (McCallum, 2005; Ouellet et al., 2003; Rachowicz et al., 2005), and *Ranavirus* (Teacher et al., 2010). Families which are declining most rapidly are Myobatrachidae (gastric brooding frogs), Leptodactylidae (Neotropical frogs), Bufonidae (true toads) and Ambystomatidae (mole salamanders) (Stuart et al., 2004). Other factors contributing to amphibian declines include the introduction of predatory species to aquatic breeding sites, especially fish (Bradford et al., 1993), global climate change (Bosch et al., 2007; Girardello et al., 2010), acidification of water (Lips, 1988), contaminants (Blaustein & Kiesecker, 2002) and increased UV-B radiation (Blaustein et al., 1997). Research indicates that the importance of any contributing factor varies depending on species, location and conditions. Therefore implementing conservation measures to protect amphibians requires a range of conservation strategies.

1.1.2. Metapopulation dynamics

Implementing effective conservation measures for declining amphibians requires an understanding of population variables such as size structure, sex ratio, survivorship and recruitment (Green, 1999). The concept of metapopulation dynamics encompasses many of these variables and been discussed widely in recent decades in relation to amphibian declines (Hamar & McDonnell, 2008; Storfer, 2003). A metapopulation can be defined as, "an assemblage of local populations inhabiting spatially distinct habitat patches" (Moilanen & Hanski, 1998), and may serve to reduce the risk of extinction in isolated populations by connecting ponds with areas of suitable terrestrial habitat to allow dispersal. Metapopulation dynamics help stabilise natural

fluctuations in population sizes and aid in identifying the causes of declining populations (Pechmann & Wilbur, 1994). Several models have been proposed to explain metapopulation dynamics. The colonisation-recolonisation theory proposed by Levins (1969) whereby the habitat consists of homogenous patches which go extinct whilst others are colonised is generally considered too simplistic with few amphibians conforming to the model (Amarasekare & Possingham, 2001; Hanski & Gyllenburg, 1993; Williams, 1999). The basis of Levin's model takes a ponds-as-patches view of amphibian spatial dynamics where ponds are regarded as populations and inter-pond movements form the metapopulation (Marsh & Trenham, 2001). Certainly amphibians do make movements between ponds (Kopecký et al., 2010; Purrenhage et al., 2009; Sinsch, 1988; Williams, 1999) and these may serve to enhance metapopulation persistence but their frequency appears to vary with species. A number of studies have used this approach in studying amphibian metapopulations (e.g. Arntzen & Teunis, 1993; Gill, 1978; Glandt, 1982; Sjogren-Gulve, 1994; Williams, 1999). However many amphibians spend large proportions of their lives in terrestrial habitat so the ponds-as-patches approach will lead to pond-based explanations for amphibian abundance and persistence (Marsh & Trenham, 2001) with little consideration of the importance of terrestrial habitat. Hanski et al., (1994) identified 4 conditions required for existence of a metapopulation under this model:

1. Habitat patches support local breeding populations.
2. No single population is large enough to ensure long-term survival.
3. Patches are not too isolated to prevent recolonisation.

4. Local patches are sufficiently asynchronous to make simultaneous extinctions of local populations unlikely.

However these conditions are rarely met and are likely to occur only in an ideal scenario (Marsh & Trenham, 2001). In a review of 53 amphibian metapopulations Smith & Green (2005) found that 33 studies took the 'ponds-as-patches' approach but events outside the ponds contributed to their dynamics. They also concluded that not all amphibians existed in a metapopulation model due to too much movement between ponds. Also local amphibian extinctions were deterministic, not stochastic, as habitats underwent succession. Alternative models have therefore been proposed including source-sink theory (Pulliam, 1988) which suggests that ponds are of unequal quality. Productive ponds which recruit are known as 'sources' and ones which are not, but absorb emigrating newts from sources, are 'sinks'. There is some evidence that shows that amphibians exist in source-sink model systems (Halley et al., 1996; Hels & Nachman, 2002; Semlitsch, 2000), but this does not always seem to be the case (Williams, 1999). Hanski (1994) proposed the incidence function model which allows simple but biologically realistic functions to describe colonisation and extinction (Lopez & Pfister, 2001). The main advantage is that this model has simple field requirements. However the study area must be completely surveyed otherwise migration ability may be overestimated. Also the model doesn't distinguish between source and sink patches (Hokit et al., 2001). As a result of these factors this model has been infrequently applied to amphibian metapopulation dynamics. Due to the general lack of clarity and consistency in determining the form metapopulations take in amphibians, it may be more

suitable to use the term in the simplest way as "a subdivided population that has a spatial structure" (Griffiths et al., 2010).

Studies which take a ponds-as-patches approach have noted large fluctuations in population size between years (Beebee, 1996; Cooke, 1995; Griffiths et al., 2010; Kupfer & Kneitz, 2000) which may to be linked to highly variable juvenile recruitment within populations (Arntzen & Tenuis, 1993). However many observed fluctuations in population size may be due to sampling error (Marsh & Trenham, 2001) or the relying solely on count data (Bailey et al., 2004b) which fail to take into account detection probabilities and lead to biased estimations of survival (Schmidt, 2005; Schmidt & Anholt, 1999). This makes it difficult to make generalisations of population parameters so conservation measures should be based on site and population-specific data. Additional research into population dynamics within different habitats and sites and utilising capture-mark-recapture data will be useful to increase our knowledge of how amphibian species may react to a changing climate.

Capture-mark-recapture studies and skeletochronological analyses have revealed that apparent annual survival of amphibians varies by species and location. Estimates of annual survival range from 0.62 in the edible frog *Rana esculenta* and 0.38 in pool frogs *R. lessonae* in northern Switzerland (Anholt et al., 2003) to 0.78 in the European tree frog *Hyla arborea* (Pellet et al., 2007). Great crested newt annual survival estimates have been calculated to range from 0.33 to 0.57 in France (Arntzen & Teunis, 1993). In two sites in

southern England annual survival estimates ranged from 0.33 to 1.0 (Baker, 1999) and 0.25 to 0.80 (Griffiths et al., 2010). Large observed variations in apparent survival may be due to temporary emigration (Bailey et al., 2004a). This behaviour has been noted in several amphibian populations including plethodontid salamanders (Bailey et al., 2004b), the fire salamander *Salamandra salamandra terrestris* (Schmidt et al., 2004) and the western toad *Bufo boreas* (Muths et al., 2006). However temporary emigration does not appear to occur in all amphibian species (Pellet et al., 2007; Schmidt & Anholt, 1999) and may explain only some of the observed fluctuations in annual survival.

Population Viability Analysis (PVA) has been performed on many amphibian populations to estimate the minimum population size. This can be defined as the smallest size required for a population or species to have predetermined probability of persistence for a given length of time (Shaffer, 1981). These values are useful for assessing conservation measures required for declining amphibians. Reed et al., (2003) concluded that approximately 7000 individuals were required for 95% chance of population persistence over 40 generations. However more recent studies suggest that a minimum number of 40 breeding females is sufficient providing the population is within 0.5km from other ponds (Halley et al., 1996). Griffiths & Williams (2000) calculated that 100 - 200 individuals may not be high enough for population persistence if ponds are isolated. Therefore it appears minimum viable population size depends on location and degree of isolation of populations.

1.1.3. Effects of predators on amphibian declines

Amphibian populations are often negatively affected by predators, especially fish (Brönmark & Edenhamn, 1994; Denöel et al., 2007; Ficetola & De Barnardi, 2004). The introduction of predatory fish has been recorded as having severely detrimental effects on amphibian populations worldwide (e.g. Crochet et al., 2004; Kats & Ferrer, 2003; Rachowicz et al., 2005). This is mainly due to direct predation on aquatic larvae. Removal of predatory fish often results in populations of amphibians re-establishing (Aronsson & Stenson, 1995), though this is often difficult to achieve and recovery may be a slow process (Kats & Ferrer, 2003). In an attempt to reduce the impacts of predators on larvae, some amphibians have been observed to avoid laying eggs in ponds which already have predators (Beebee, 2007; Manenti et al., 2009; Orizaola & Brańa, 2003). Also, larvae of some amphibian species exhibit behavioural or morphological phenotypic plasticity when in the presence of predators. These include reducing movement or altering microhabitat use which may reduce predation risk (Kiesecker & Blaustein, 1997; Van Buskirk & McCollum, 2000), or developing broader tails to allow faster swimming (Van Buskirk, 2002). However not all amphibians exhibit such plasticity in responses, especially if larvae are faced with a new predator (Semlitsch, 1988; Laurila & Aho, 1997). Further research is required to ascertain the degree to which that amphibians exhibit these responses in natural environments.

1.1.4. Effects of climate on amphibian declines

In a globally changing climate the impacts on amphibians are still relatively poorly understood (Blaustein et al., 1994b; Pechmann & Wilbur, 1994; Salvidio, 2009). Research has indicated that the impacts on species will vary depending on ecology and location (Girardello et al., 2010). Evidence suggests that changing climatic conditions will impact synergistically with other factors including UV-B radiation and diseases to impact on populations (D'Amen & Bombi, 2009; Lips et al., 2006). For example the western toad *Bufo boreas* in North America appears to experience egg survival of up to 50% due to the synergistic effects of UV-B, pathogenic fungi and lower water levels due to changes in climate (Kiesecker & Blaustein, 1995). Worldwide, declines have been attributed to the "climate-linked epidemic hypothesis". This predicts that amphibian populations, particularly in tropical regions, will decline in unusually warm years due to changes in disease dynamics (Pounds et al., 2006). This has also been confirmed in Europe where Bosch et al. (2007) examined 28 years of data on 10 amphibian species within central Spain. Bosch et al. (2007) concluded that an increase in temperature and mists during the breeding season promoted the growth of *Batrachochytrium dendrobatidis*, the causative agent of the disease chytridiomycosis, resulting in a rise in infected species.

Climate change within the UK is predicted to bring hotter, drier summers and mild, wet winters (Defra, 2009). A long-term study on the common toad *Bufo bufo* in southern England indicates that females emerging from hibernation after milder winters have lower body condition, which may impact on survival

and reproductive ability (Reading, 2007). In a study in Kent, male great crested newts *Triturus cristatus* exhibited a lower estimated annual survival after milder winter minimum air temperatures (Griffiths et al., 2010). However studies which examine the effects of climate on amphibians are relatively few due to the length of time required to implement. Further research into this area is required over future decades.

1.1.5. European perspectives

Within Europe, 23% of amphibian species are currently in decline (IUCN, 2011a). All species which are classed as threatened (Critically Endangered, Endangered or Vulnerable) are endemic to Europe (Temple & Cox, 2009) which highlights the importance of research and conservation to protect these species. The main causes for declines in Europe are habitat loss, pollution and introduced species, particularly fish (Temple & Cox, 2009). In common with global amphibian declines, multiple factors may act together to potentially affect the reproductive success and survival of species in different habitats (Nyström et al., 2007). Of the seven native amphibian species in the UK, four species either have a restricted distribution or declining status (Baker et al., 2011). These are the natterjack toad *Epidalia* (*Bufo*) *calamita*, northern pool frog *Pelophylax* (*Rana*) *lessonae*, great crested newt *Triturus cristatus* and common toad *Bufo bufo*. Two of these (natterjack toad and great crested newt) receive full protection under the European Habitats Regulations, 1992 and national Wildlife and Countryside Act, 1981. Causes of declines in these species are mainly due to pond loss as a result of urban

development, lowering of water tables, modern farming practices, lack of management, pollution and agrochemicals (Clemons, 1997).

1.2. The great crested newt *Triturus cristatus*

1.2.1. Classification and description

The class Amphibia is divided into three orders: Anura (frogs and toads), Caudata (newts and salamanders) and Gymnophiona (caecilians). The family Salamandridae (Caudata) contains small to medium sized terrestrial or aquatic salamanders which are found within both Old and New Worlds. The genus *Triturus* currently comprises 7 species of large-bodied newts, including *Triturus cristatus*, the great crested or warty newt (AmphibiaWeb, 2011).

Great crested newts grow up to 170 mm in length and possess dark, granular skin with white stippling along both flanks. The ventral surface is brightly coloured orange/yellow with distinctive black markings. During the breeding season males develop a jagged crest along the back that dips at the rear of the abdomen and have a white stripe down the tail that fades as it approaches the body (Langton et al., 2001). Males possess a relatively more swollen cloaca (vent) than females. Females remain dark brown/black and possess a bright orange ventral tail stripe which remains throughout the year. Aquatic larvae grow external, feathery gills at the base of the head and possess a distinctive tail with black spots and a filament at its tip (Figure 1.1). Juveniles resemble small females with black, warty skin and a yellow/orange underside.

Figure 1.1. Larval great crested newt showing gills, tail spots and filament.

1.2.2. Distribution and status

Great crested newts inhabit a wide range across central France, Britain, southern Scandinavia and through Central Europe to the Ural Mountains and Western Siberia (Jehle et al., 2011). Within the British Isles great crested newts have been recorded in Scotland, Wales and England (Steward, 1969). Population densities are highest within central and eastern counties of England, but scarcer in the north and west. A national survey by Swan & Oldham (1993) revealed that great crested newts were present in 2.5% of ponds and ubiquitous within 30% of counties, with the highest densities in lowland areas.

Despite its widespread distribution throughout Europe great crested newts are thought to be declining in 11 countries (Corbett, 1994) and is one of Europe's fastest declining amphibian species (Griffiths, 2001). The species is listed on Appendix II of the Bern Convention, 1979 and Annexes II and IV of the European Habitats Directive, 1992. This requires European countries to provide Special Areas of Conservation (SAC) for great crested newts. Within the British Isles, great crested newts are also listed on Schedule 5 of the

Wildlife and Countryside Act, 1981 which prevents the deliberate disturbing, taking, killing or injury of this species or its habitat; offering for sale or to significantly affect its ability to survive, breed or nurture their young (The Conservation of Habitats and Species Regulations, 2010).

Within the British Isles it is estimated that the great crested newt declined at a rate of between 0.5% and 4% a year during the period 1960 to 1990 (English Nature, 2001a). The 70% decline in dew ponds on the Sussex downs in the period 1950 - 1977 led to a large reduction in populations of great crested newts (Beebee, 1977a) partly due to the species' poor ability to colonise new areas and its vulnerability to the presence of sticklebacks (Beebee, 1977b). In the 1980s, 70% of counties experienced great crested newt declines (Oldham & Swan, 1997) and 85% of suitable habitat was lost due to ponds filling or silting up, urban development and improved drainage (Cooke & Scorgie, 1983). In recent years declines have been attributed to habitat destruction and fragmentation, pollution, pond drainage and changes in agricultural practices (Clemons, 1997; Wood et al., 2003).

1.2.3. General biology

Adults return to breeding ponds in February/March having spent the winter months in terrestrial habitats. Males usually arrive at breeding ponds before females (Sullivan et al., 1995) where they develop secondary sexual characters including crest and white tail stripe (Griffiths & Mylotte, 1988). Males defend temporary leks in areas of open water, in which they perform a complex mating display to attract females (Hedlund & Robertson, 1990). If

successful, a courting male will deposit a spermatophore on the pond substrate which the female collects into her vent, resulting in internal fertilization. Females may mate with more than one male across the breeding season, which lasts until June. Once fertilized, females lay between 200 and 300 eggs wrapping each individually within a leaf (Miaud, 1995), a process which may take many weeks. Larvae hatch after two to three weeks (Langton et al., 2001) and feed on zooplankton and other invertebrates. In the first few weeks after hatching, larvae tend to be nektonic, floating within the water column. After a further two to three months larvae change their behaviour to become more benthic and secretive (Dolmen, 1983). Metamorphosis generally occurs late summer/early autumn (August to October). Newly metamorphosed juveniles leave their natal ponds to find suitable terrestrial habitats such as woodland, long grass or scrub. Breeding adults may remain in ponds for several months before leaving to spend the autumn and winter (October to March) in terrestrial habitats which may be under logs, stones, within leaf litter or soil (Jehle & Arntzen, 2000). Juveniles spend between two and four years on land during which they may return to water to feed (Verrell & Halliday, 1985).

1.2.4. Microhabitat preferences

1.2.4.1. Aquatic habitats

Great crested newts breed in a wide variety of aquatic water bodies in many habitat types including: farmland, woodland, dune slacks and gravel pits (Beebee, 1996). In general they appear to be more exacting in their aquatic habitat preferences than other newt species by inhabiting deeper, more

secluded ponds (Griffiths, 2001). Preferred ponds are in the size range 500 to 750 m^2 with individuals often avoiding small garden ponds (Oldham & Swan, 1997) and larger water bodies which may contain fish (Swan & Oldham, 1993). Emergent and submergent aquatic vegetation (50 to 75% cover) are important for egg laying and providing a refuge for larvae and adults (Griffiths & Mylotte, 1987). The presence of fish appears to be a major factor in reducing or eliminating populations (Baker & Halliday, 1999; Beebee, 1981, 1997; Skei et al., 2006). Larvae are particularly vulnerable due to their nektonic behaviour which increases their vulnerability to predation (Joly et al., 2001). Ponds which desiccate occasionally remove fish predators and may therefore contain higher populations of great crested newts. Other optimal habitat requirements include: low numbers of breeding water fowl, good terrestrial habitat within 500 m and several ponds in a 1 km^2 radius (Oldham et al., 2000).

Within aquatic habitats great crested newts prefer certain microhabitat conditions. During the day adults tend to seek dark, benthic areas whilst at night, particularly in the breeding season, individuals utilise open water microhabitats (Dolmen, 1983). Inter-specific dietary niche partitioning appears to occur in ponds with great crested newts consuming larger prey than smooth or palmate newts (Griffiths & Mylotte, 1987). Great crested newts remain highly faithful to breeding sites (Beebee, 1996; Werner et al., 2007) and rarely move between ponds (Williams, 1999) though they may exhibit repeated movements back and forth between land and ponds during

the breeding season (Latham & Oldham, 1996). Therefore colonisation of new ponds may be achieved mainly by juvenile dispersal (Beebee, 1996).

1.2.4.2. Terrestrial habitats

In addition to aquatic habitats, great crested newt adults and juveniles occupy a range of terrestrial habitats for feeding and growth. Our ecological understanding of the use of terrestrial habitats by great crested newts lags behind that of the aquatic environment (Schabetsberger et al., 2004; Malmgren, 2007; Oldham et al., 2000) due to their secretive habits and low detection. Observations of great crested newts on land show that individuals favour habitats which provide undisturbed damp and shady conditions (MacGregor, 1995). These include deciduous woodland, scrub and rank grassland (Latham & Oldham, 1996). Studies on the related Italian crested newt *Triturus carnifex* using radio-tracking devices attached to individuals has revealed that newts occupy a range of microhabitats including small mammal burrows (72% of individuals), under rocks (20%) and inside rotting tree stumps (Schabetsberger et al., 2004). Other studies involving great crested newts in France showed that individuals utilised areas under leaf litter (44%), within small mammal burrows (34%), in leaf litter (13%) and under logs (6%) (Jehle & Arntzen, 2000). During colder weather newts may also bury themselves up to 30 cm underground to avoid the frost (Watson, 1994).

Adults may move for several hundred metres on land, often showing preference for particular habitats e.g. woodland/scrub. On leaving breeding

habitats, many individuals head for bare soil, possibly indicating the importance of underground habitats for hibernation (Jehle & Arntzen, 2000). Within a one year period individuals may move up to a 1 km (Jehle & Arntzen, 2000) though many individuals probably do not migrate this far (Kupfer & Kneitz, 2000; Laan & Verboom, 1990). Jehle (2000) found that 50% of those individuals studied only moved a few metres from the pond and some may rarely go further than the pond edge (Baker, 1999).

1.2.4.3. Juveniles

Aquatic larvae metamorphose into juveniles which leave their natal ponds to spend the first years of life predominantly in terrestrial habitats. Compared to other aspects of great crested newt ecology the terrestrial habitat use of juveniles is poorly understood, especially in the period after leaving natal ponds (Malmgren, 2007). Most experimental research has concentrated on patterns of juvenile emigration and the cues used in orientation (Hayward et al., 2000; Hayward, 2010; Jehle & Arntzen, 2000). Emigrating juveniles appear to follow the scent of adults (Hayward, 2010) moving towards favourable habitats such as woodland, scrub and rough grasses. Individuals may roam over 800m during their first year of life (Kupfer & Kneitz, 2000) spending their time feeding and growing. The microhabitats utilised seem to be similar to those used by adults and include areas in woodland, scrub and rough grassland. Studies of other amphibians suggest that juveniles are more wide ranging in their movements than adults (Breden, 1987) and migrate far enough to encounter other ponds (Sinsch, 1997). Great crested newt growth rates are fastest in juveniles and appear to slow as individuals

mature (Raymond & Hardy, 1990). Growth rates vary among individuals so body size is a poor predictor of age (Hagstöm, 1979; Verrell & Halliday, 1985). Other aspects of juvenile ecology such as population dynamics and the effects of climate change remain largely unknown, requiring the need for further research.

1.2.4.4. Impacts of predation

Adult and juvenile great crested newts possess glands in the skin which are distasteful to predators (Griffiths, 1996). Aquatic larvae are particularly vulnerable to predation due to their lack of toxicity and nektonic behaviour (Joly et al., 2001). Predation risk by fish poses a particular risk to larval great crested newts and may severely affect populations (Beebee, 1981, 1996; Horton & Branscombe, 1994; Skei et al., 2006). There is some evidence to show that adult great crested newts avoid breeding in ponds which contain fish (Beebee, 2007; Malmgren, 2003) resulting in reduced pond occupancy. The species of fish present may have differential effects on great crested newts. Crucian carp *Carassius carassius* are bottom detritus feeders (Tonn et al., 1994) and have minimal impact on great crested newts. In contrast sticklebacks *Gasterosteus aculeatus* are active free-swimming hunters and readily consume larvae, resulting in severe impacts on great crested newt populations (Cooke, 1994; McLee & Scaife, 1992).

1.3. Project aims

Although several aspects of great crested newt ecology have been studied, there are still many areas which are little understood, a greater

understanding of which will aid in conservation of this declining species. The overall aim of this study is to increase our knowledge and understanding of microhabitat use and ecology of great crested newts within a woodland area in the British Isles. The following specific aims will be examined as part of this study:

1. To examine the population structure and metapopulation dynamics of great crested newts within a woodland area.

2. To investigate variations in body condition index, apparent annual survival and growth rates of terrestrial juvenile great crested newts.

3. To examine the effect of winter climatic conditions on body condition in great crested newts and asses impacts on apparent annual survival.

4. To determine the effect of body condition on crest height in male great crested newts.

5. To assess the impacts of caged predatory fish on the survival of embryos and hatching morphology in the great crested newt.

6. To determine the behavioural responses of great crested newt larvae when in the presence of predatory fish.

7. To examine the effects of intra-specific competition on oviposition behaviour in great crested newt females.

1.4. Chapter summaries

Chapter 2. Study sites and general methods

The study area consisting of four ponds are described along with general methods for capturing and identifying adult and juvenile great crested newts.

Chapter 3. Population dynamics of the great crested newt *Triturus cristatus* within a woodland area

Many population studies on amphibians rely on count data which may fail to take into account detection probabilities and lead to biased estimations of survival (Schmidt, 2005; Schmidt & Anholt, 1999). There have been few long-term studies on great crested newt sub-populations which examine these parameters (Griffiths et al., 2010). This study over a five year period in four ponds used capture-mark-recapture techniques and population modelling to generate estimates of apparent annual survival and detection probabilities. A greater understanding of these parameters will aid our understanding of how great crested newt populations may be affected by changes in the environment and climate.

Chapter 4. Ecology of juvenile great crested newts *Triturus cristatus* in their terrestrial phase

The ecology of terrestrial juveniles is poorly understood (Malmgren, 2007) with few studies examining this phase in the life history of great crested newts. This study aims to elucidate: how juveniles utilise terrestrial habitats; differences in growth rates; population dynamics over a four year period; how winter temperature affects body condition and survival. An increased

understanding of juvenile ecology will be important for providing further conservation measures for this species.

Chapter 5. Factors affecting the body condition in a great crested newt *Triturus cristatus* population

Several studies have examined how body condition in great crested newts varies with individual age and sex (Cooke & Arnold, 2003) or at times of the year (Arntzen et al., 1999; Baker, 1992). Few studies have examined how winter temperatures may affect body condition in great crested newts over more than three years. This study assesses the impacts of winter temperature on body condition in a population of great crested males over a five year period. Findings will be useful for understanding how a changing climate may affect great crested newt populations.

Chapter 6. Secondary sexual characters as honest indicators of fitness in male great crested newts *Triturus cristatus*

Lowered body condition may result in reduced crest height in male great crested newts (Baker, 1992). Research has indicated that males with larger crests have higher mating success (Baker & Halliday, 2000). However no research has examined how variations in body condition affect male crest height over a longer time period and in several populations. This study examined how male body condition may impact on crest height in a population over a five-year period in three ponds, and suggests possible implications of findings on reproduction in the great crested newt.

Chapter 7. Non-consumptive effects of predatory three-spined stickleback *Gasterosteus aculeatus* on great crested newt *Triturus cristatus* embryos

The direct impacts of fish on survival of great crested newt populations such as consumption of larvae are well documented (Beebee, 1981, 1996; Horton & Branscombe, 1994; Skei et al., 2006). However no studies have examined if any indirect or non-consumptive effects of sticklebacks occur on great crested newts whilst in the embryonic stage. Experiments carried out in this study explored the impacts of caged predatory sticklebacks on great crested newt embryos and determined whether hatching larvae show phenotypic plasticity in developmental timing or morphology which may serve to decrease predation risk.

Chapter 8. Non-consumptive effects of caged predatory fish on the behaviour of great crested newt *Triturus cristatus* larvae

It is understood that great crested newt larvae are vulnerable due to their lack of toxicity and nektonic behaviour (Dolmen, 1983). However no studies have determined whether larvae alter their microhabitat usage in an attempt to reduce predation risk. Experiments carried out in this chapter examined the behaviour of great crested newt larvae when faced with caged non-predatory crucian carp and predatory stickleback, both in relation to degree of movement and microhabitat choice.

Chapter 9. The effects of intra-specific competition on oviposition behaviour in female great crested newts *Triturus cristatus*

Survival of eggs is crucial for the persistence of any aquatic breeding amphibian species. Eggs laid on unsuitable substrate or in poor environmental conditions may lead to lower survival which may ultimately affect species' reproductive success (Sih & Maurer, 1992; Spence et al., 2007). Depositing eggs close to those of conspecifics may have positive outcomes due to safety in numbers and decreasing chances of predation or cannibalism (Iwai et al., 2007). Alternatively, laying eggs close to others may lead to an increased spread of fungal infections (Green, 1999) or competition for resources such as nutrients or oxygen. No study has determined the choices of female great crested newts in relation to intra-specific competition on oviposition sites. This study seeks to determine whether great crested newt females avoid laying eggs on substrate which already has eggs attached. The outcomes will have implications for individual female fitness and how this may affect reproductive success.

Chapter 10. Conclusions

The main aim of this project was to increase our knowledge and understanding of great crested newt ecology in a woodland area. Implications for our understanding of the ecology of this species and its conservation will be discussed in light of results obtained from each area of study.

Chapter 2. Study sites and general methods

2.1. Study sites

2.1.1. Epping Forest description and location

Epping Forest lies to the northeast of London stretching more than 19 kilometres in an arc from Wanstead in the south to Epping in the north, covering approximately 2,430 hectares (May et al., 2000). Pollen analysis reveals a history of continuous woodland cover since the Neolithic period, c. 3,000 BC (Brimble, 1991). During the past 2,000 years the Forest has been extensively used for human activities, notably cattle grazing, woodland pasture and as a Royal Hunting Forest (City of London, 2007). Since 1878 the Forest has been owned and managed by the City of London Corporation who have reintroduced some of the traditional management techniques. Over two-thirds of the Forest is designated a Site of Special Scientific Interest and a Special Area of Conservation under European legislation (City of London, 2007). The geology consists of London clay and claygate beds with localised cappings of bagshot beds and pebble gravel (Layton, 1985). Approximately two-thirds of the Forest consists of deciduous woodland, predominantly common beech *Fagus sylvatica* on lighter soils with pedunculate oak *Quercus robur* and European hornbeam *Carpinus betulus* on the heavier clays (Brimble, 1991). In addition there are considerable areas of secondary woodland, predominantly silver birch *Betula pendula*. Other habitats of note are acid grassland and lowland heath both of which provide valuable habitats for wild flowers, butterflies and reptiles (Brimble, 1991). There are approximately 100 ponds within Epping Forest, all of which are man made.

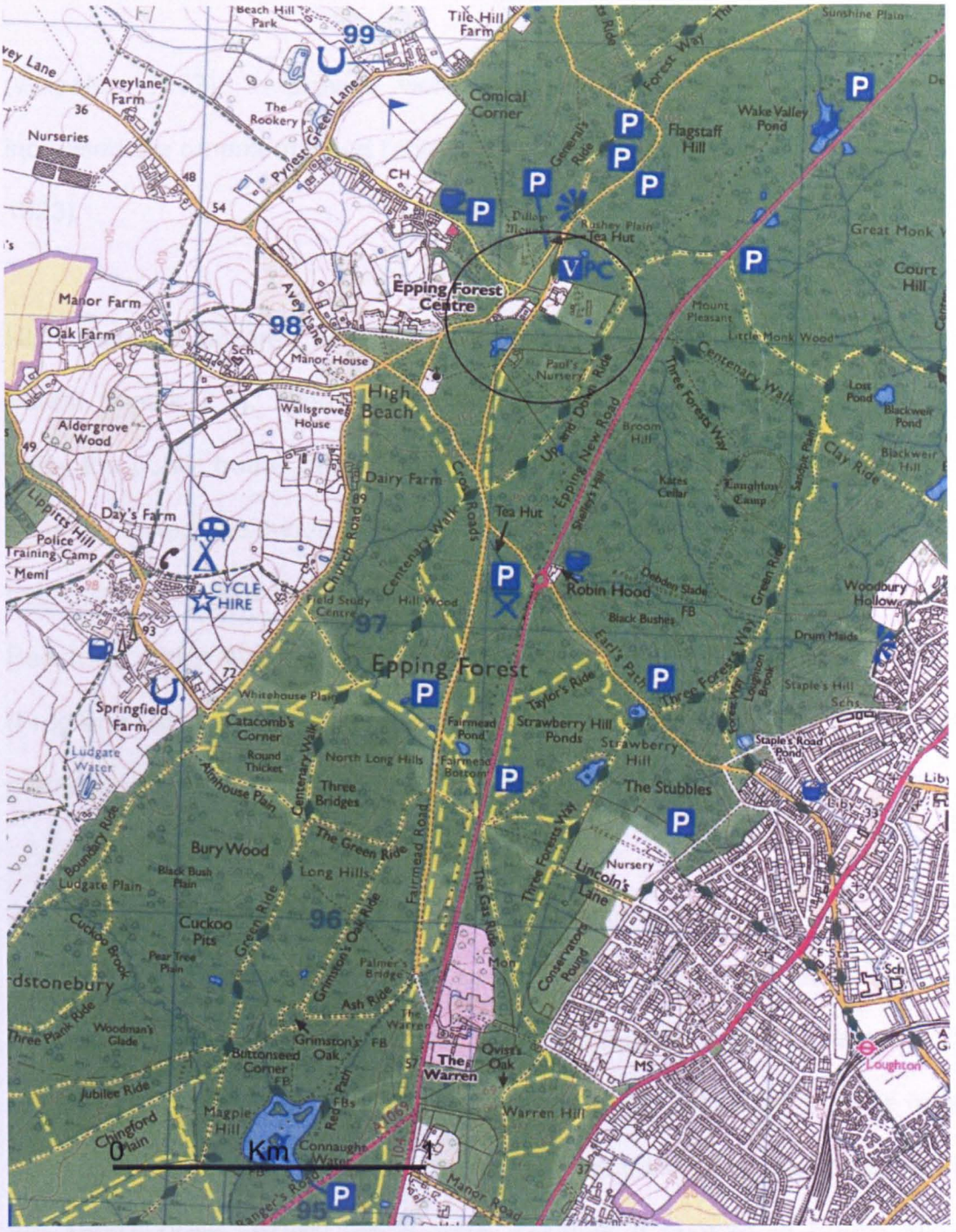


Figure 2.1. The northern part of Epping Forest showing the study site at High Beach. Map reproduced from Ordnance Survey map for Corporation of London, 2002. 1:20,000.

These were formed as a result of: gravel extraction, post 1879; the need to provide water for cattle during times of grazing; bombs dropped by the Luftwaffe in 1940's creating depressions which later filled with water

(Wheeler, 1992); to provide work for local unemployed people which increased the ornamental and recreational facilities within the Forest (Buxton, 1923).

High Beach is an area of approximately 80 ha in the northern part of the Forest (Figure 2.1). Thousands of tourists visit High Beach each year to enjoy the public house, tea huts, car park and open grassland of the Pillow Mounds. Mixed deciduous woodland consisting of predominantly silver birch, common beech and hornbeam surround this area. Four ponds lie at High Beach, of which two are within the grounds of Epping Forest Field Centre (Frog and Lily ponds). Speakman's and Oak Plain ponds lie several hundred metres apart set within mixed deciduous woodland (Figure 2.2).

2.1.2. Status of great crested newts within Epping Forest

During the middle of the twentieth century surveys revealed that the great crested newt had a "sparse and scattered" distribution within Epping Forest (Yalden, 1967) and was found in only a few ponds. These low records may have been due to the species' secretive and secluded behaviour because in the 1980's great crested newts were found at a number of Forest sites including several hundred individuals in a swimming pool at High Beach (Pickett, 1986). A more recent study revealed great crested newt presence in 29% of the 94 Forest ponds surveyed (Corporation of London, 2000).

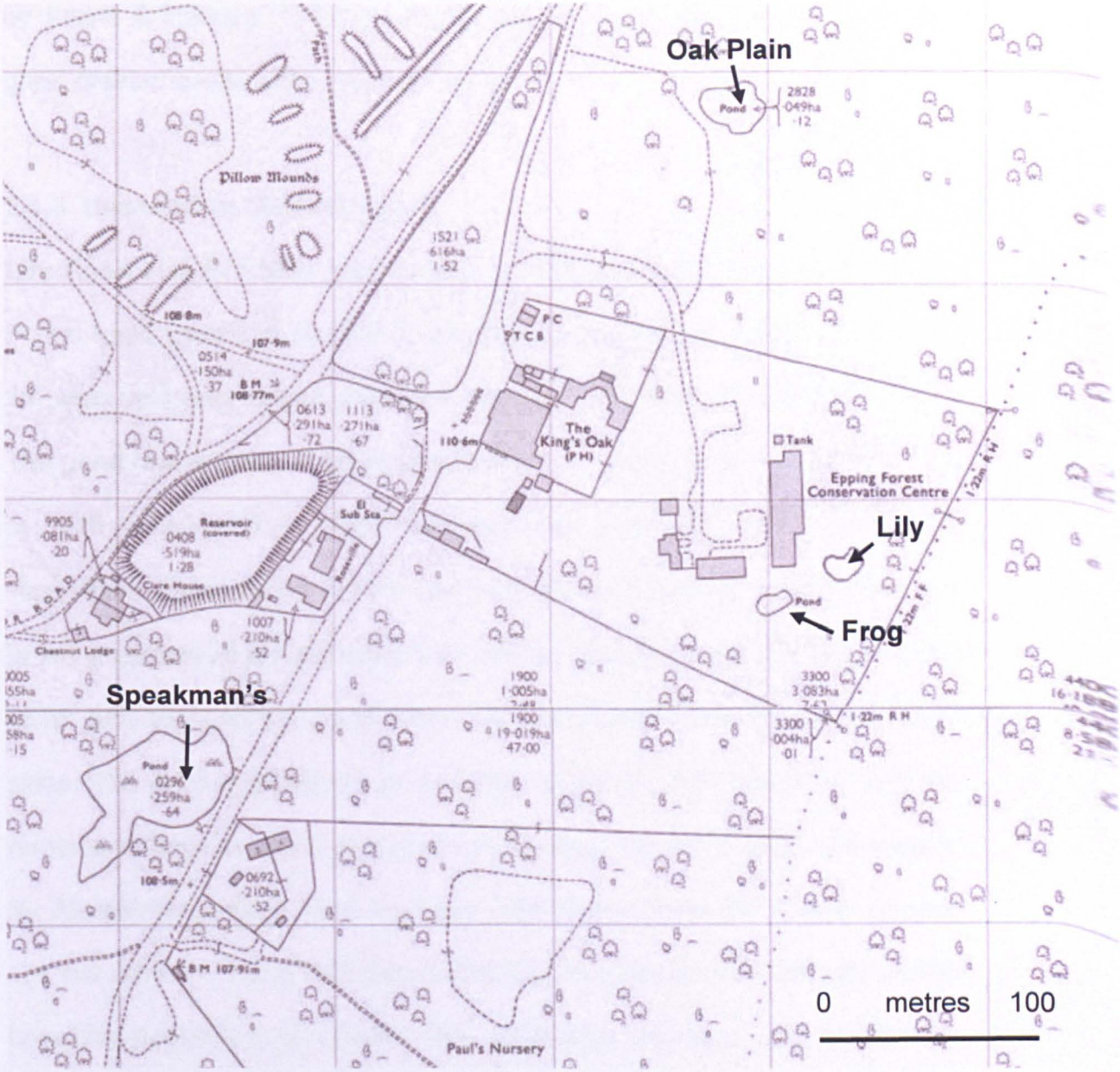


Figure 2.2. High Beach showing Field Centre and four study ponds. Map reproduced from Ordnance Survey map of High Beach, 1972. 1:1,250.

However introductions of fish into many Forest ponds have had negative impacts on great crested newt populations with reports of poor recruitment and survival from previously successful sites (Corporation of London, 2002). Within the ponds at High Beach, Frog and Oak plain ponds were described

by Atkins & Herbert (1998) as being "one of the most important ponds for great crested newts within Epping Forest".

2.1.3. Description of study ponds

Lily Pond (TQ 413 980) was created in 1998 within the grounds of Epping Forest Field Centre to provide an additional resource for visiting students. A 1m wide perimeter gravel path provides clear and easy access (Figure 2.3). The pond has an area of approximately 120 m^2 and a maximum depth of 1.3 m with approximately 60% coverage of emergent and submergent vegetation. Water levels remain relatively constant throughout the year due to the presence of a butyl pond liner. Mixed deciduous woodland lies to the south and east, with a small lawn and field centre buildings on the other sides. The pond vegetation was partially cleared in 2006 and 2011 with the removal of large amounts of nuttall's pond weed *Elodea nuttallii*, white water lily *Nymphaea odorata* and bogbean *Menyanthes trifoliata*. These species are still common along with yellow flag iris *Iris pseudacorus*. An established breeding population of crucian carp *Carassius carassius* has existed for many years and remained for the duration of the study.

Figure 2.3. Lily Pond in the summer showing perimeter path to allow easy access for students to carry out freshwater invertebrate studies.

Frog Pond (TQ 413 980), created in 1971, lies 26m to the west of Lily Pond within the grounds of Epping Forest Field Centre. Five sets of gravel steps allow easy access to the water which is approximately 2 m below ground level (Figure 2.4). The pond has an area of approximately 110 m^2 and a maximum depth of 1.3 m with a clay lining. Water levels drop considerably in dry periods but the pond rarely dries out completely due to periodic addition of tap water from the Field Centre. Between the steps along all banks grow large densities of yellow flag iris and bramble *Rubus spp.* In 2007 bur-reed *Sparganium erectum* covered approximately 60% of the pond surface but by 2011 this had grown to over 85% coverage along with water soldier, which appeared in 2009. The abundance of these two species was reduced by pond clearance in 2011. In addition, duckweed *Lemna minor* and floating sweet-grass *Glyceria fluitans* grow occasionally throughout the pond. To the south, east and west lies deciduous woodland; the Field Centre buildings and lawn are 5 m to the north.

Figure 2.4. Frog Pond in winter (left) and summer (right).

Oak Plain Pond (TQ 412 982) formed after gravel extraction to build local roads. The close proximity of deciduous woodland results in limited access,

especially during the summer months, due to low overhanging branches. A public footpath, bridleway and open grassy area nearby provide access for students and visitors to Epping Forest. The pond has an area of approximately 490 m^2 and maximum depth of 1 m, although due to the thick layer of detritus, this may be increased by a further 40 cm. Water levels fall rapidly in the summer months resulting in desiccation once or twice a decade. Abundant floating sweet-grasses (*Glyceria spp.*) form dense mats between one and two metres wide around the pond margins as the year progresses. In summer this species may cover up to 70% of the water surface along with smaller amounts of nuttall's pond weed and white water lily (Figure 2.5).

Figure 2.5. Oak Plain Pond in winter (left) and summer (right).

Speakman's Pond (TQ 410 979) is a highly ephemeral water body with annual desiccation (Figure 2.6). To aid in water retention in the summer months the City of London dug 1 m wide and 0.8 m deep trenches around the pond base (Figure 2.7). Although relatively large at 2,600

Figure 2.6. Speakman's Pond (winter).

m^2, access to the water edge is extremely limited due to dense growth of over-hanging goat willow (*Salix caprea*). A relatively busy minor road lies adjacent to the south eastern edge of the pond providing access to a popular public house and car park approximately 600 m to the northeast. Pond water levels reach a maximum of 1.2 m from the natural pond bottom and up to 1.8 m from the base of the trenches. This pond once held a high diversity of aquatic plants including lesser spearwort *Ranunculus flammula*, water crowfoot *R. peltatus*, and purselane *Portulaca oleracea* but since the accidental addition of Australian swamp stonecrop *Crassula helmsii*, diversity has been greatly reduced due to extensive formation and spread of large, dense mats across the whole pond.

Figure 2.7. Speakman's Pond when dry (left). Note over-hanging willows. Trenches within the pond area (right) retain water for longer periods.

2.2. General methods

2.2.1. Sampling methodology

Adult great crested newts were captured from the four study ponds using hand-made funnel traps. These consisted of 2-litre plastic drinks bottles with the lids inverted (as described in Griffiths, 1985) (Figure 2.8). In Frog, Oak

Plain and Speakman's ponds, bamboo canes with flagging were used to secure traps (Griffiths & Langton, 2003). Due to the butyl liner in Lily Pond, traps were secured to the sides of the banks using string and weights. Care was taken to ensure air was caught in each trap to provide a supply of oxygen for any trapped newts (Griffiths & Langton, 2003).

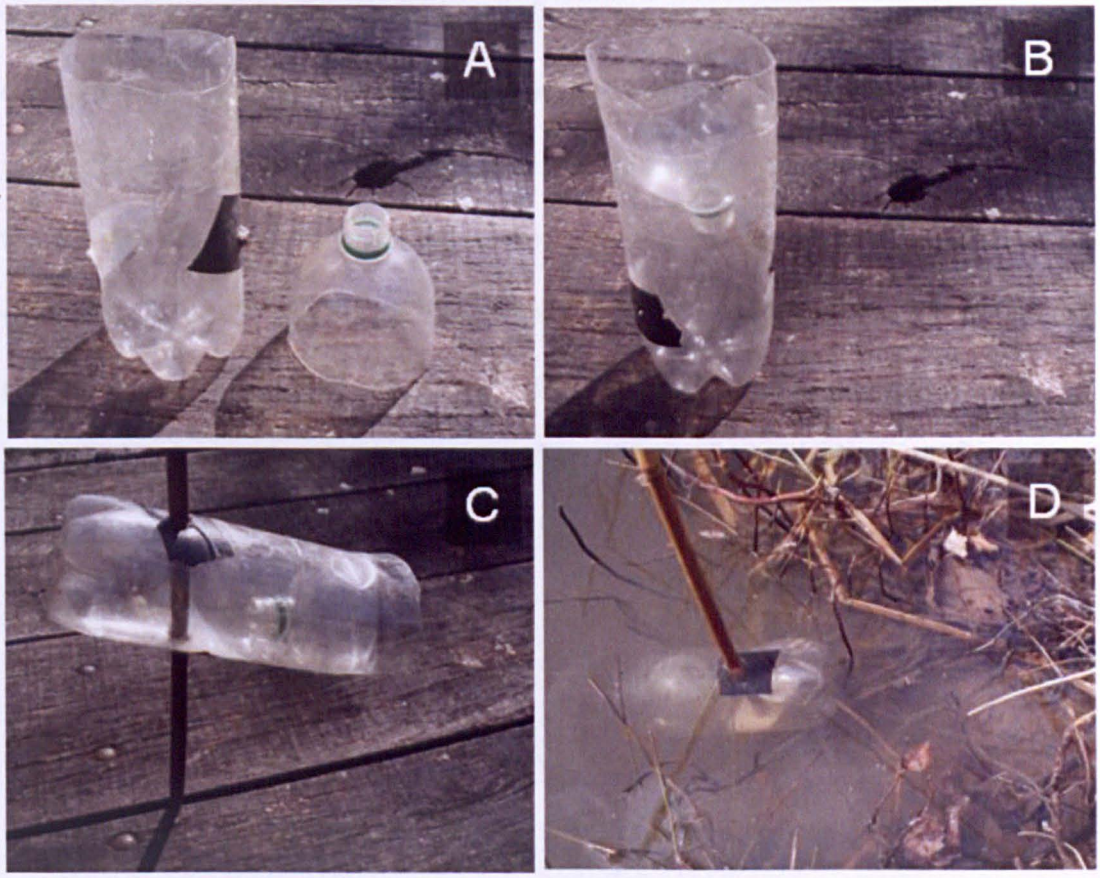


Figure 2.8. Photographs showing the construction of a funnel trap. A. Two-litre plastic drinks bottle with lid cut off; B. Lid is inverted and inserted into the top of the cut bottle; C. Bamboo cane is inserted through two small holes cut into the bottle. The slight slant helps trap a reservoir of air at the top of the trap for newts to breathe; D. Traps are placed into the pond margins facing the centre at 2 m intervals.

Trapping commenced in the last week of February and finished in the last week of June each year 2007 to 2011. These were set twice a week in each pond for the period 2007 to 2011. This resulted in a total of 36 trapping occasions in each pond per year. Over the five year duration of the project, 180 trapping occasions were conducted in each of the four ponds. Traps were placed at the same point in each pond, with positions marked by natural markers e.g. logs, trees. Artificial markers were not used because of potential disturbance/removal by either members of the public or students using ponds for dip netting studies. Traps in all ponds were set at standard 2 m intervals (Griffiths & Inns, 2003) around each pond margin after 17:00 h and positioned with the open end pointing towards the pond centre and left overnight. All traps were removed before 08:00 h the following morning. Due to variations in pond size, accessibility and water depth, slightly different numbers of traps and positioning was used in each pond.

Frog Pond: A total of 20 traps were set per night at a distance of approximately 0.5 m from the shore using bamboo canes. Trapping success varied with a mean of 1.67 great crested newts captured per night in 2011 compared to 5.44 per night in 2008. Mean numbers of smooth newts *Lissotriton vulgaris* in traps varied from 2.58 per night in 2011 to 4.86 in 2007. Palmate newts *L. helveticus* were encountered most frequently in traps with 2.19 per night in 2011 to 8.39 per night in 2008. Details of trapping success can be seen in Table 2.1.

Lily Pond: Due to its smaller size, 18 traps were set per night, floating at a distance of between 0 and 0.2 m from the pond edge, secured with string attached to weights on the bank. Mean numbers of great crested newts per night were lower compared to Frog pond, ranging from 0.19 in 2011 to 3.97 in 2007. The mean number of smooth and palmate newts in traps was approximately equal and similar in magnitude to that of great crested newts. Mean numbers of smooth newts in traps ranged from 0.75 newts per night in 2011 to 1.53 in 2009. The lowest number of palmate newts captured per night was in 2011 with only 0.5 individuals captured, compared to a maximum of 2.17 individuals per night in 2008 (Table 2.1).

	Trapping success per year (number of newts per pond per session)	Frog	Lily	Oak Plain	Speakman's
2007	Lv	4.86	1.19	7.81	0.00
	Lh	5.28	1.00	18.39	0.00
	Tc	5.00	3.97	0.33	0.00
2008	Lv	2.69	1.17	6.08	0.00
	Lh	8.39	2.17	12.03	0.00
	Tc	5.44	1.31	4.31	0.00
2009	Lv	4.11	0.75	3.33	2.25
	Lh	8.39	1.67	9.53	2.75
	Tc	2.11	0.50	2.28	0.75
2010	Lv	3.44	1.53	4.39	1.75
	Lh	4.47	1.11	9.42	1.75
	Tc	3.47	0.22	2.67	1.00
2011	Lv	2.58	0.67	3.06	0.00
	Lh	2.19	0.50	2.33	0.00
	Tc	1.67	0.19	0.47	0.00

Table 2.1. Mean numbers of great crested, smooth and palmate newts captured per pond per trapping attempt in each year. Lv = Lissotriton vulgaris *(smooth newt), Lh* = L. helveticus *(palmate newt), Tc* = Triturus cristatus *(great crested newt).*

Oak Plain Pond: A total of 40 traps were set per night in Oak Plain Pond due to its larger size. The majority of the pond perimeter was accessible but there was a stretch of approximately 10 m where overhanging trees prevented placing of traps. Traps were placed 1.0 m from the pond edge to deter members of the public reaching and removing traps. The mean number of great crested newts captured per night ranged from 0.33 in 2007 to 4.31 in 2008. Mean numbers of smooth and palmate newt captured per night were considerably higher than the other two ponds. Captures of smooth newts ranged from 3.06 per night in 2011 to 7.81 in 2007. Palmates were recorded in highest abundance with a minimum of 2.33 captured per night in 2011 to a maximum of 18.39 in 2007 (Table 2.1).

Speakman's Pond: This pond had a shoreline perimeter which exceeded the number of traps available. Therefore 60 traps were placed along three x 40 m stretches of pond shoreline. In addition, access to the pond edge was difficult due to overhanging willow trees (*Salix caprea*). Traps were placed at between 0 and 1.0 m from the pond edge, depending on accessibility. This pond had the least number of captures with no newts being caught in 2007, 2008 and 2011. In the remaining years, only between 0.75 and 1.0 great crested newts were captured per night. Smooth and palmate newts were also captured in low numbers with a mean of between 1.75 and 2.25 individuals of either species being caught per trapping night (Table 2.1).

All newts captured in traps were identified and sexed using secondary sex characters. Numbers of each sex of smooth and palmate newt were recorded

and individuals released at point of capture. All great crested newts captured were taken to an outside work station located by the pond of capture. Snout-vent length (SVL) was measured to the nearest 0.5 mm using callipers. Tail depth was used as an index for crest height since the latter is difficult to measure accurately (Griffiths & Mylotte, 1988). Tail depth was measured by holding newts firmly and placing the tail along a ruler, reading to an accuracy of ± 0.5 mm. Weight was recorded to the nearest 0.01 g using digital pocket scales. To record belly markings individuals were placed on a small plastic transparent plate with raised sides and a sponge gently placed over the top to provide a uniform background. A compact digital camera was used to take photographs of belly pattern markings from underneath. Newts were not turned upside down or restrained as this resulted in strong squirming movements, leading to blurred photographs (personal observation). If picked up gently and placed down, newts often remained still for a few seconds (personal observation). This allowed enough time to take an accurate record of the belly pattern from underneath the transparent plate. Once all measurements and data had been collected individuals were released into the pond of capture. This was usually within 1 hour of removal of traps from the pond.

2.2.2. Great crested newt belly pattern recognition

Each individual great crested newt has a unique belly pattern marking (Arntzen et al., 1999) which allows individual newt recognition (Griffiths, 2001). Images of belly pattern markings were resized and rotated using Microsoft® Picture Manager to ensure a consistent orientation, and stored as

jpeg files. Images were saved into separate computer folders depending on sex and year. Images were not sub-divided into pattern groups since there was a large range in markings, making categorization difficult. To correctly identify a new individual, the belly pattern image was compared to thumbnail images of previously captured newts in Microsoft Windows® Explorer. The new individual was compared to all images within its sex group for each year until a match was found. If there was no previous recording of the individual it would be saved as a 'new newt' and given a unique code (sex followed by date of capture and identification number e.g. F29040914) and placed in the correct sex and year category computer folder. If a match was found, the individual would be saved as a 'recapture' and given a unique code representing how many times it had been captured previously (e.g. F29040914R4). This ensured all images of newts were carefully identified and stored for accurate data analysis.

2.2.3. Weather data

Mean minimum daily air temperature and rainfall were recorded at an official on-site meteorological weather station maintained by Epping Forest Field Centre on behalf of the UK Meteorological Office.

Chapter 3. Population dynamics of the great crested newt *Triturus cristatus* within a woodland area

3.1. Abstract

A capture-mark-recapture study of a great crested newt metapopulation over five years was conducted within a two-hectare area of deciduous woodland. A total of 1,289 newts were captured from four ponds in the breeding seasons 2007 to 2011. Incidence of recapture varied between ponds and years in the range 6.9 to 66.3%. Model selection based on Akaike's Information Criterion indicated that apparent annual survival varied with sex but remained constant with site and year. Apparent annual survival was 0.40 and 0.58 for males and females respectively. Detection probabilities fluctuated widely between years and ponds between 0.0 and 0.68. Only 11 inter-pond movements were recorded in five years, representing 0.85% of the captured metapopulation. Estimates of population size varied considerably between pond and year. Total estimates ranged from 100 to 2,113 individuals in five years. Sex ratios of 1:1 were recorded in two study ponds. One pond was dominated by males for two years and females for three whilst in the last pond females outnumbered males by 2:1 or higher in all years. Results from this study highlight the need for detection probabilities to be calculated in studies involving amphibian populations to avoid biases in estimates of apparent survival and population sizes.

3.2. Introduction

Effective conservation of declining amphibians requires a thorough understanding of population dynamics (Alford & Richards, 1999). This includes knowledge of metapopulation dynamics, the effects of climate change and gene exchange on populations (Carey & Alexander, 2003). Many studies on amphibians have demonstrated that populations fluctuate over time (Collins & Storfer, 2003; Pellet et al., 2006; Richter & Seigel, 2002), however a large proportion of these rely on count data to estimate population demographics and do not take into account detection probabilities (Schmidt, 2003). This leads to inaccurate estimates of annual survival and population size which may misinform conservation strategies (MacKenzie et al., 2004; Weidong & Swihart, 2004). Amphibians have detection probabilities of less than one (Schmidt, 2005) and detection probabilities vary across space and time (Bailey et al., 2004d; Dodd Jr. & Dorazio, 2004). A failure to determine this parameter is a major fault in many population studies (Bailey et al., 2004c) resulting in unreliable estimates of population size and survival (MacKenzie et al., 2004). This may have consequences for the conservation of declining amphibians. The information-theoretic approach to building and selecting models (Burnham & Anderson, 2002) has been applied to estimating population parameters in a range of amphibian, bird and mammal species in recent years (e.g. Angelini et al., 2010; Ozgul et al., 2006; Wright et al., 2009; Zeh et al., 2002). The use of Akaike's Information Criterion (AIC) in model selection is of value as it provides an effective method for selecting suitable models (Lebreton et al., 1992). However application of these techniques to amphibian species is relatively rare (Bailey et al., 2004b). More

research on amphibian population dynamics is required, utilising the information-theoretic approach of model selection to take into account detection probabilities and make more realistic estimates of annual survival and population size. This will give greater understanding of population dynamics and enable more effective conservation of declining species (Schmidt, 2005).

Many population studies on the great crested newt have been carried out (e.g. Arntzen, 2000; Baker, 1999; Griffiths et al., 2010; Kupfer & Kneitz, 2000; Hagström, 1979) but in general, many of these have either relied on simple count data (e.g. Cooke, 1986; Cooke 1995) or have not fully taken detection probabilities into account (e.g. Cooke & Arnold, 2003). Studies which have incorporated capture-mark-recapture techniques reveal that great crested newts exhibit large fluctuations in population size between years. Hagström (1979) observed a variation from 67 to 205 individuals over two consecutive years and Miaud et al. (2001) recorded large variations between ponds with estimates varying from 10 to 179 individuals. Similarly, Miaud et al. (1993) found that population sizes doubled over one year and noted population extinction over one breeding season. This was due to the introduction of predatory catfish which consumed larvae and triggered movements of adults to nearby ponds. High levels of variation in larval recruitment may explain population fluctuations (Arntzen & Teunis, 1993; Griffiths et al., 2010; Kupfer & Kneitz, 2000). Alternatively variations in adult annual survival may also be responsible for population variation (Schmidt, 2005).

Temporary emigration is the process where adults remain away from breeding ponds for a year and are thus unavailable for capture (Muths et al., 2006). This behaviour has been documented in many amphibian populations (Kendall et al., 1997; Sandercock, 2006; Schmidt et al., 2002). If temporary emigration occurs then capture probabilities will be underestimated, the estimated number of individuals at the breeding site will be positively biased and values will have larger confidence intervals (Bailey et al., 2004b; Muths et al., 2006; Kendall et al., 1997; Schaub et al., 2004). However not all amphibian populations exhibit temporary emigration (e.g. Schmidt et al., 2007; Schmidt & Anholt, 1999; Williams, 1999) and this behaviour appears to depend on local processes and conditions.

Population studies on amphibians reveal considerable variation in annual survival (Cooke & Arnold, 2003; Gill, 1978). Hagström (1979) estimated relatively high annual survival (78%) in a great crested newt population. Adults generally experience higher survival than juveniles, often varying between years. In three separate studies, Baker (1999) found annual survival ranged from 31 to 100%, Arntzen & Teunis (1993) observed survival of between 33 and 57% and Griffiths et al., (2010) estimated values at between 25 and 80%. Annual survival is likely to be affected by a range of factors including habitat quality (Lecis & Norris, 2003; Sinsch, 1988), distance from nearby ponds (Halley et al., 1996) and climatic conditions (Reading, 2007). The different levels of survival indicated by these studies makes it increasingly apparent that few generalisations can be made about population

survival. Research must become more population- as well as species-specific (Griffiths et al., 2010).

The sex ratio at breeding ponds is crucial in population persistence since a shortage of females may result in poor mating success and lowered larval recruitment. Many studies report a balanced sex ratio of 1:1 in breeding amphibians (Arntzen & Teunis, 2003; Hagström, 1979; Miaud et al., 1993), however in a number of populations males are more prevalent than females (Blackwell et al., 2004; Gill, 1978; Halley et al., 1996; Hedlund, 1990; Kopecký et al., 2010; Sexton et al., 1990), particularly at the beginning of the breeding season (Arntzen, 2002). This is because males are more aquatic at breeding ponds and more available for capture (Arntzen, 2002). The sex ratio in common toads *Bufo bufo* may change through the breeding season (Sullivan et al., 1995) with males arriving earlier than females. In newts it appears that females may be more numerous at breeding ponds (Andreone & Giacoma, 1989) and this may be due to egg-laying in aquatic vegetation (Verrell & Halliday, 1985).

Great crested newts are known to be highly site faithful, returning to favoured breeding ponds each year (Beebee, 1996). Terrestrial movements may include dispersal and movements between ponds (Denöel et al., 2007; Purrenhage & Niwiarowski, 2009). Some movements between ponds are required for metapopulation persistence (Hanski & Gyllenburg, 1993), but too many will lead to breakdown in the metapopulation structure (Smith & Green, 2005) resulting in a metacommunity (Parris, 2006) or collection of patchy

populations (Petranka & Holbrook, 2006). In a study in Kent, Williams (1999) noted only 8 individuals make inter-pond movements between years, representing a small proportion of the individuals captured. However this level of movement was sufficient to maintain metapopulation persistence. Other amphibian species may make more numerous movements between patches including the alpine salamander *Mesotriton alpestris* (Kopecký et al., 2010), the edible frog *Rana esculenta* (Peter, 2001) and spotted salamander *Ambystoma maculatum* (Purrenhage et al., 2009). In these cases the species were living in patchy populations, resulting in different inter-pond dynamics. Several studies show that great crested newts live in metapopulations (e.g. Miaud et al., 1993; Williams, 1999) but further studies of great crested newts using capture-mark-capture techniques are required to fully understand metapopulation processes in this species.

This chapter aims to:

- Record the fluctuations in incidences of capture in four great crested newt ponds over a five year period.
- Use an information theoretic approach in program MARK to estimate population parameters of apparent survival and detection probabilities within a great crested newt metapopulation.
- Determine the sex ratio of breeding adults at ponds.
- Establish the degree of inter-pond movements and discuss implications for metapopulation dynamics.

3.3. Methods

3.3.1. Study sites

Four ponds at High Beach, Epping Forest were sampled twice weekly, February to July, 2007 to 2011 (see Chapter 2 for details). Due to an unexpected water leak from an adjacent Thames Water reservoir in spring 2009, trapping in Speakman's Pond became hazardous due to high water levels. Water levels remained high throughout the breeding season, limiting the number of traps which could be set. No data are available for Speakman's Pond for the 2011 breeding season due to an exceptionally dry spring resulting in complete pond desiccation by mid-April.

3.3.2. Sampling methodology

Sampling was carried out as described in Chapter 2.

3.3.3. Habitat Suitability Index

A great crested newt Habitat Suitability Index (HSI) score was calculated for each pond and its surrounding area in 2009 (Oldham et al., 2000). This calculation is based on 10 habitat variables which are given a Suitability Index (SI) score (see Appendix 1 for details of each SI variable). These are between 0 and 1 and are based on data and graphs produced by Swan & Oldham (2000). The HSI calculation is thus:

$$SI_1 \times SI_2 \times SI_3 \times SI_4 \times SI_5 \times SI_6 \times SI_7 \times SI_8 \times SI_9 \times SI_{10}^{1/10}$$

Where SI = the SI score for each habitat variable. The closer the HSI score is to 1.0, the more suitable the habitat for great crested newts. HSI scores were calculated for each pond.

3.3.4. Overview of Capture-Mark-Recapture (CMR)

3.3.4.1. Cormack-Jolly-Seber model and assumptions

The Cormack-Jolly-Seber (CJS) model is a restricted version of the Jolly-Seber (JS) model which is applied to open populations. The former uses a maximum likelihood approach to calculate apparent survival (Φ) and probability of detection (p) (Schaub et al., 2004). Since deaths may be confounded with permanent emigration (Mazerolle et al., 2007), the term apparent survival is used (Pledger et al., 2003). The assumptions of the CJS model are (Mazerolle et al., 2007):

1. Individuals are homogenous in survival and detection probabilities.
2. There is no emigration from the study area.
3. Samples are instantaneous and animals are released immediately after sampling.
4. Marks are not lost, and all marks are read correctly.

Assumptions (3) and (4) are likely to be met in great crested newt studies since all animals are released after sampling and marks cannot be lost since recognitions is by photography of belly pattern markings (Arntzen, 2000). However there was a small chance of observer error in recognising belly patterns (Andreone, 1986). The likelihood of this occurring was reduced in this study by dividing the recorded markings into sub-groups resulting in fewer individuals to compare. Assumption (1) is more difficult to fulfil, but amphibians exhibit less variation in individual patterns of behaviour than groups such as birds or mammals and are therefore ideal organisms for this

type of study. Adult amphibians are extremely site faithful (Beebee, 1996) so permanent emigration is less likely to be a problem. Temporary emigration may occur but due to time and resource constraints it was not possible to determine whether this behaviour was taking place in this study. Findings will be discussed in light of this limitation.

3.3.4.2. Program MARK

Program MARK (White & Burnham, 1999) utilises the live-recapture CJS model and uses the maximum likelihood approach to build and select models (Burnham & Anderson, 2002). In this study two important parameters were calculated: apparent annual survival (Φ) and detection probabilities (p). Population data were encoded into binary code where '1' indicated presence and '0' absence in each sampling year. Thus a capture history was created for each individual newt: 00100 indicates that an individual was absent in the first two sampling years, present in the third and absent from the last two. In addition covariate groups were added which were sex and pond.

3.3.4.3. Building and selecting models; estimation of adult survival and detection

The information-theoretic approach was utilised to create models with varying numbers of parameters based on Φ and p. The global model with the largest number of parameters was: {Φ (time + sex + pond), p(time + sex + pond)} i.e. apparent survival and detection probabilities vary with time, sex and pond. Models with fewer parameters were then derived from this global model. Goodness-of-fit to the global model was performed by parametric

bootstrapping of 2000 iterations. Akaike's Information Criterion (AIC) was used to perform model selection. AIC makes an estimate of the expected distance between the fitted model and the unknown, true data (or reality) (Burnham & Anderson, 2002). The principle of parsimony was used in model selection i.e. the model with the best fit to the data using the lowest number of parameters. Delta QAICc (ΔQAICc) corrected for small sample size was used to give a measure of each model relative to the best fitting model. Models ranked with the lowest ΔQAICc were considered to best support the data (Burnham & Anderson, 2002). Values for ΔQAICc < 2.0 were regarded as having considerable support for the data. ΔQAIC of between 3.0 and 7.0 had little support and values > 10 data were unlikely to support the data (Burnham & Anderson, 2002). Over- or under- dispersion can lead to biases in survival and detection probabilities (Schmidt et al., 2002). Therefore the variance inflation factor, ĉ was applied to model selection. This was calculated by dividing the deviance from the global model from that of the mean of the bootstrap models (White & Cooch, 2012). A value of ĉ = 1, indicates no over dispersion, < 1 = under- dispersion and > 1.0 suggests that over dispersion may be occurring (Mazerolle, 2006).

3.3.4.4. Estimation of population size

Population size was calculated by dividing the number of individuals captured at a particular pond in a given year by 1/detection probability for that year (Griffiths et al., 2010).

3.4. Results

3.4.1. Habitat Suitability Index scores

Speakman's Pond had the lowest HSI score of 0.59 due to the high level of shoreline shade and annual desiccation. Frog, Lily and Oak Plain ponds all had relatively high HSI scores (0.84, 0.80 and 0.92 respectively), indicating they are all suitable for great crested newts. Oak Plain scored 1.0 for each SI with the exception of pond count within a 1 km radius. This indicates that Oak Plain pond has very high suitability for great crested newts. Frog Pond's score at 0.84 was also high but its slightly small size and high level of permanence reduced the HSI value. Lily Pond had a slightly lower score than Frog and Oak Plain ponds due to its minor population of fish (Table 3.1).

Frog

SI	1: Location	2: Pond area (m^2)	3: Pond desiccation	4: Water quality	5:% shade	6: No. fowl	7: No. fish	8: Pond count	9: Terrestrial	10: Macrophyte cover	Score
Score	A	170	Never	Good	40%	None	None	5	Excellent	70%	
S.I. Value	1	0.4	0.9	1	1	1	1	0.5	1	1	**0.8424**

Lily

SI	1: Location	2: Pond area (m2)	3: Pond desiccation	4: Water quality	5:% shade	6: No. fowl	7: No. fish	8: Pond count	9: Terrestrial	10: Macrophyte cover	Score
Score	A	170	Never	Good	40%	None	Slight	5	Excellent	60%	
S.I. Value	1	0.4	0.9	1	1	1	0.66	0.5	1	0.9	**0.7997**

Oak Plain

SI	1: Location	2: Pond area (m2)	3: Pond desiccation	4: Water quality	5:% shade	6: No. fowl	7: No. fish	8: Pond count	9: Terrestrial	10: Macrophyte cover	Score
Score	A	450	Occasional	Good	40%	None	None	5	Excellent	60%	
S.I. Value	1	1	1	1	1	1	1	0.5	1	0.9	**0.9233**

Speakman's

SI	1: Location	2: Pond area (m2)	3: Pond desiccation	4: Water quality	5:% shade	6: No. fowl	7: No. fish	8: Pond count	9: Terrestrial	10: Macrophyte cover	Score
Score	A	1200	Annual	Medium	100%	Minor	None	5	Excellent	80%	
S.I. Value	1	0.9	0.1	0.66	0.2	0.8	1	0.5	1	1	**0.5857**

Table 3.1. Habitat Suitability Index scores for the four study ponds.

3.4.2. Capture overview

3.4.2.1. Number of captures

A total of 1,289 newts were captured during the breeding seasons 2007 to 2011. There was high variability in captures between ponds and years (Figure 3.1). Total captures ranged from 424 in 2008 to 47 in 2011. All ponds showed a similar pattern of captures across years with the highest numbers in 2008 and lowest in 2011. Numbers of captures ranged from three to 25 individuals in Lily, 17 to 131 in Frog, 11 to 155 in Oak Plain and 0 to 6 in Speakman's Pond.

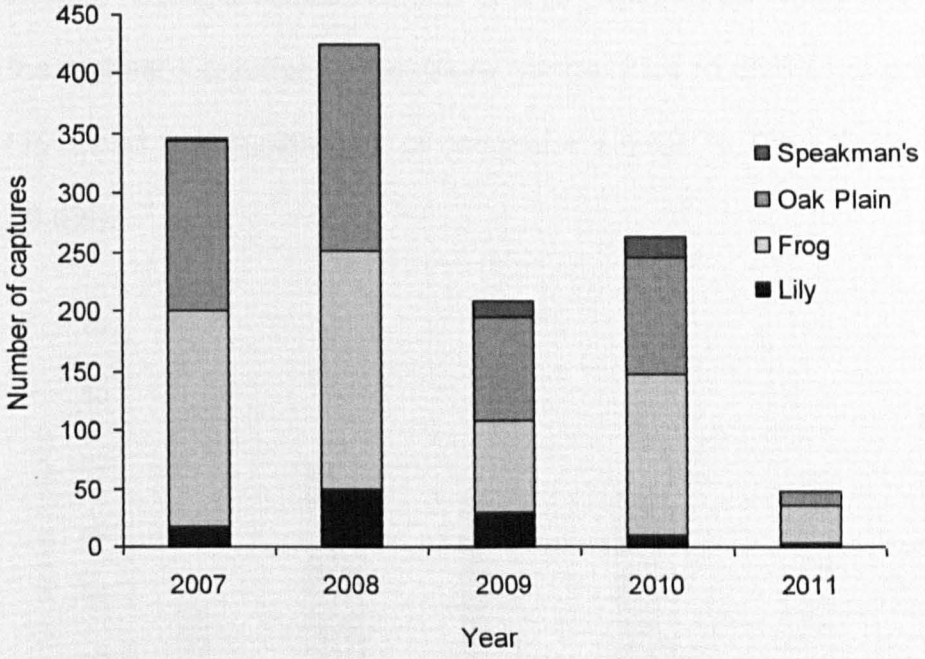


Figure 3.1. Number of captures of great crested newts in funnel traps for each pond, 2007 - 2011.

Frog Pond had the highest number of captures in all years except 2009. Numbers of great crested newts captured from Oak Plain Pond were of the same magnitude as Frog Pond in all years except 2009. The number of captures in Lily Pond was low in all years. Despite its close proximity to Frog Pond (26 m), the highest number of newts caught in Lily Pond was 50 in 2008, compared to 201 in Frog Pond in the same year.

Speakman's Pond had no captures in 2007, 2008 and 2011 with only 31 caught over the remaining years.

3.4.2.2. Recaptures

The percentage of great crested newts recaptured was highly variable between ponds and years (Figure 3.2). No individuals were recaptured from Speakman's Pond, which is likely to be due to the low number of initial captures. Oak Plain Pond had a low incidence of recapture in all years (range: 6.9 to 16.0%), despite having a similar number of total captures as Frog Pond. Frog Pond had the highest incidence of recapture (range: 28.4 to 66.3%) of ponds in all years. Lily Pond had incidences of recapture similar to Frog Pond (range: 17.6 to 50.0%).

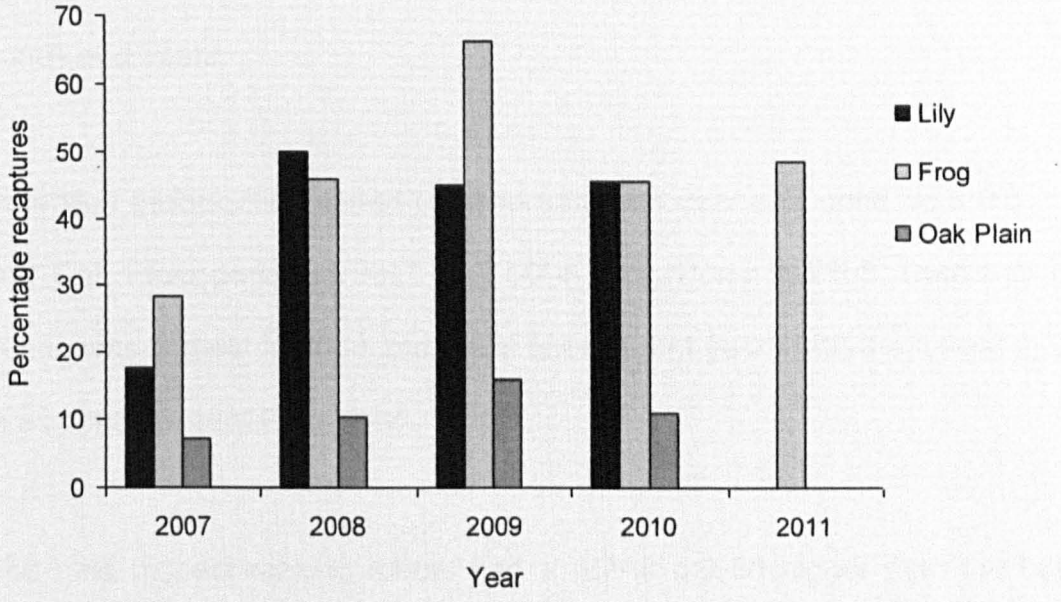


Figure 3.2. Percentage recaptures of great crested newts in each pond, 2007 to 2011. There were no recaptures from Speakman's Pond.

Percentage recaptures increased considerably from 2007 to 2009 in all ponds (except Speakman's). Recaptures peaked in 2009 after which they fell to a low in 2011 (except Frog Pond).

3.4.3. Metapopulation survival and detection

Data from all years and ponds were pooled and analysed in program MARK. Results from 2000 bootstrap iterations showed there was over-dispersion in the data so model selection was corrected using the variance inflation factor, ĉ to a value of 1.2717. The most parsimonious model {Φ(sex), p(time + pond)} indicates that apparent annual survival varies with sex and that detection probabilities vary with time and pond (Table 3.2). Apparent annual survival was estimated at 0.40 (C.I. = 0.29 - 0.51) and 0.58 (C.I. = 0.45 - 0.70) for males and females respectively (Table 3.3). Apparent survival was constant between ponds and years.

Detection probabilities varied considerably with year and pond from 0.0 in Lily and Oak Plain ponds in 2011 to 0.60 in Frog Pond in 2008. Detection in all ponds was highest in 2008 and fell in subsequent years with the lowest in 2011 in all ponds except Frog Pond.

The next highest ranking model had a ΔQAICc 2.88 higher than the highest ranking model, indicating considerably less support for this compared to the most parsimonious model data (Table 3.2). Therefore this and lower ranking models need not be considered in analysis.

Model	QAICc	Δ QAICc	QAICc weights	Number of parameters
Φ(sex),p(time + pond)	708.36	0.00	0.62284	14
Φ(time + pond),p(sex + pond)	711.25	2.88	0.14738	18
Φ(time + pond),p(sex)	711.83	3.46	0.11018	14
Φ(.),p(time + pond)	714.19	5.82	0.03387	13
Φ(time),p(pond + sex)	714.66	6.30	0.02670	10
Φ(sex + pond),p(time + pond)	715.55	7.19	0.01710	18
Φ(time + sex),p(time + pond)	715.76	7.39	0.01544	19
Φ(pond),p(time + pond)	717.39	9.03	0.00681	15
Φ(time),p(time + pond)	717.76	9.40	0.00567	15
Φ(time + pond),p(pond)	719.32	10.96	0.00260	15
Φ(time + pond),p(.)	720.35	11.99	0.00155	13
Φ(time + sex),p(pond)	720.56	12.20	0.00140	11
Φ(.),p(time + sex + pond)	720.74	12.38	0.00128	25
Φ(time + sex),p(sex + pond)	721.00	12.64	0.00112	14
Φ(time + pond),p(time + sex)	721.29	12.93	0.00097	19
Φ(sex),p(pond)	721.31	12.95	0.00096	5
Φ(.),p(sex + pond)	722.05	13.69	0.00066	7
Φ(sex),p(time + sex + pond)	722.79	14.43	0.00046	26
Φ(sex),p(sex + pond)	723.10	14.73	0.00039	8
Φ(time),p(time + sex + pond)	723.61	15.25	0.00030	27

Table 3.2. CJS model selection showing the 20 most highly ranked models for five years of data based upon ΔQAICc in program MARK. Φ = survival, p = detection probability. QAICc = quasi Akaike Information Criteria adjusted using ĉ.

			95% Confidence Interval	
Parameter	Estimate	Standard Error	Lower	Upper
1: Φ male	0.3988528	0.0573182	0.2934272	0.5145714
2: Φ female	0.5763009	0.0652754	0.4461213	0.6966863
3: p Frog 08	0.6016878	0.0962809	0.4073555	0.7685094
4: p Frog 09	0.3794653	0.0794487	0.2399183	0.5422716
5: p Frog 10	0.3440447	0.1018349	0.1780301	0.5594940
6: p Frog 11	0.3757771	0.1045229	0.2008678	0.5904575
7: p Lily 08	0.2644477	0.1555325	0.0697734	0.6327935
8: p Lily 09	0.5261036	0.1630354	0.2355593	0.7999850
9: p Lily 10	0.1060350	0.0854039	0.0198834	0.4095063
10: p Lily 11	0.0000000	0.0000001	-0.0000001	0.0000001
11: p OP 08	0.1351894	0.0541366	0.0593337	0.2792356
12: p OP 09	0.1021262	0.0384592	0.0476123	0.2055827
13: p OP 10	0.0619856	0.0330582	0.0212224	0.1676356
14: p OP 11	0.0000000	0.0000000	0.0000000	0.0000000

Table 3.3. Model output for pooled data from program MARK showing real function parameters of most parsimonious model: {Φ(sex),p(time + pond)}. Standard error and confidence intervals corrected for ĉ = 1.59.

3.4.4. Inter-pond movements

Only 11 inter-pond movements were recorded in the five year study period (Table 2.4). Four of these were from Frog to Lily ponds and three in the opposite direction. Only two movements were recorded between Frog and Oak Plain ponds and none between Lily and Oak Plain ponds. This represents just 0.85% of the captured population.

Year	Frog to Lily	Lily to Frog	Frog to OP	OP to Frog	Lily to OP	OP to Lily
2007-08	4	0	0	0	0	0
2008-09	1	1	2	0	0	0
2009-10	1	2	0	0	0	0
2010-11	0	0	0	0	0	0

Table 3.4. Number of movements recorded between ponds in the period 2007 to 2011. OP = Oak Plain Pond.

3.4.5. Estimation of population size

Estimates of population sizes show large variations between ponds and years (Figure 3.3). Total estimates ranged from 100 newts in 2011 to 2,113 in 2010. All ponds, with the exception of Lily Pond, had the lowest estimates in 2011 and the highest in 2010. Lily Pond had the lowest population estimates throughout the five years with a maximum of 192 individuals in 2008. The lowest value of 3 individuals in 2011 may be due to low recaptures and extremely unreliable estimates of detection probabilities. Frog Pond had a population range of 400 great crested newts in 2010 to 87 in 2011. Oak Plain appeared to have the highest population estimates which were highest in 2010 at 1,613 individuals and lowest in 2011 at just 11.

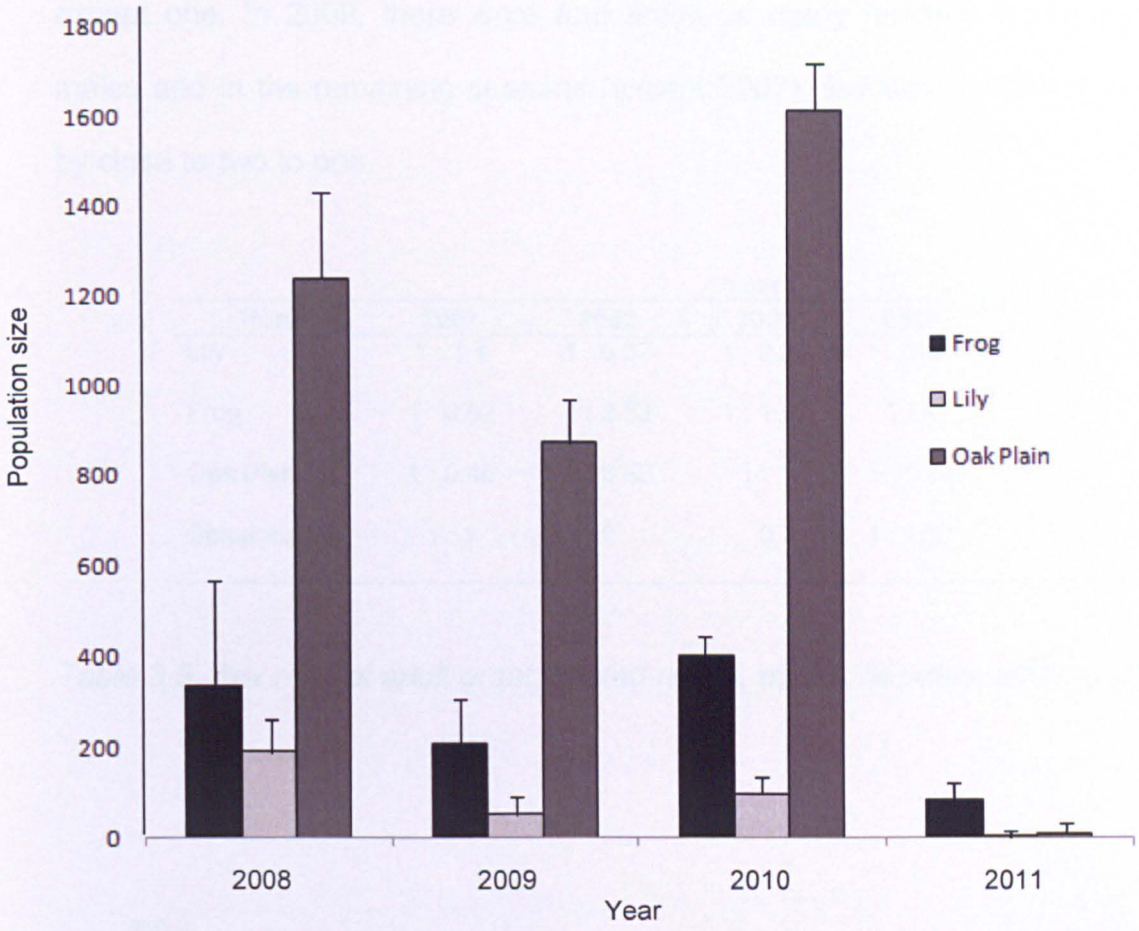


Figure 3.3. Estimated population size with standard error of great crested newts in three study ponds, 2008 - 2011.

3.4.6. Sex ratio

There was high variability in the sex ratio between ponds and years (Table 3.5) (Figure 3.4). Captures from Speakman's Pond indicate a 1:1 male to female sex ratio but these findings are likely to be unreliable due to the low number of captures. Males dominated females in two out of five breeding seasons in Oak Plain Pond. Between 2010 and 2011 females dominated males by nearly two times. Lily Pond showed a variable sex ratio which fluctuated close to 1:1. There was no distinct annual pattern with the dominating sex often changing annually. This may be due to the low numbers of total captures which may have biased the overall sex ratio. Frog Pond was dominated by females in all years

except one. In 2009, there were four times as many females captured than males and in the remaining seasons (except 2007), females dominated males by close to two to one.

	Year				
Pond	**2007**	**2008**	**2009**	**2010**	**2011**
Lily	1 : 1.4	1 : 0.57	1 : 0.29	1 : 0.88	1 : 1.33
Frog	1 : 0.82	1 : 2.38	1 : 1.92	1 : 4	1 : 2
Oak Plain	1 : 0.46	1 : 0.53	1 : 1	1 : 1.82	1 : 1.7
Speakman's	1 : 1	0	0	1 : 1.57	0

Table 3.5. Sex ratio of adult great crested newts, males: females, 2007 to 2011.

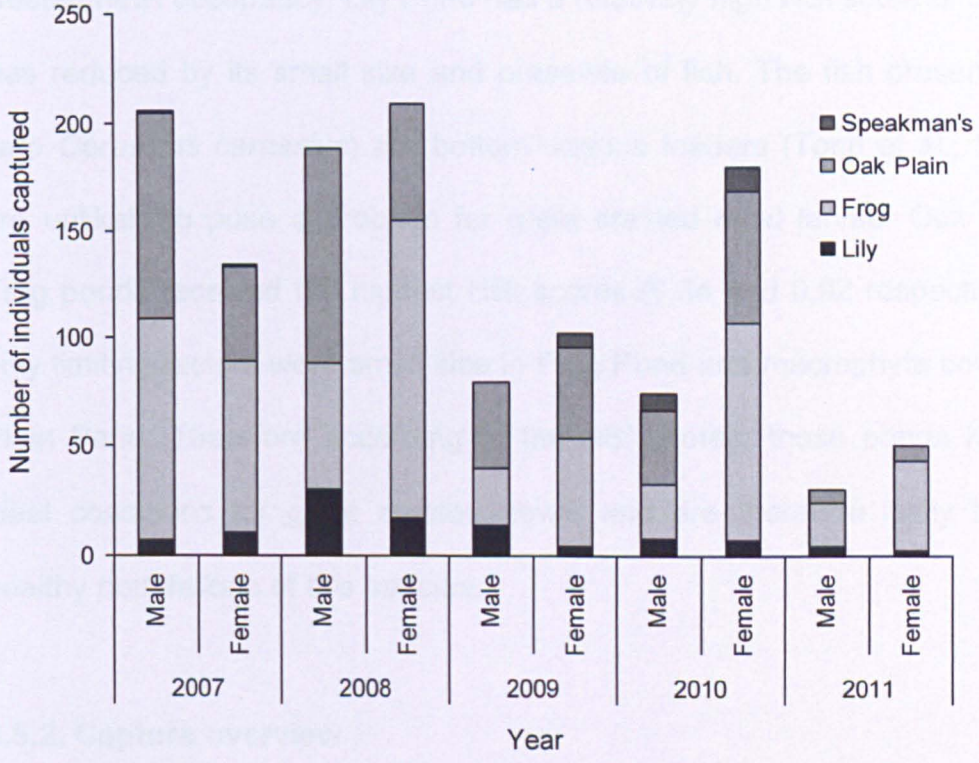


Figure 3.4. Number of individual male and female great crested newts captured, 2007 to 2011.

3.5. Discussion

3.5.1. Habitat Suitability Index scores

Speakman's Pond had the lowest HSI score of 0.59 which was mainly due to 100% shoreline shade and annual desiccation. Shoreline shade counteracts the growth of macrophytes, which limits egg-laying opportunities and increases the organic content of ponds leading to eutrophication (Oldham et al., 2000). This is likely to reduce the reproductive success of great crested newts. Periodic desiccation is beneficial in reducing aquatic predators such as fish and dragonfly larvae (McLee & Scaife, 1992). However annual desiccation may be detrimental as great crested newt larvae remain aquatic until late September or early October (Fasola & Canova, 1992) so early drying can lead to larval mortality. Due to these factors Speakman's Pond has a low likelihood of great crested newt occupancy. Lily Pond had a relatively high HSI score of 0.80 which was reduced by its small size and presence of fish. The fish present (crucian carp *Carassius carassius*) are bottom detritus feeders (Tonn et al., 1994) and are unlikely to pose a problem for great crested newt larvae. Oak Plain and Frog ponds received the highest HSI scores (0.84 and 0.92 respectively). The only limiting factors were small size in Frog Pond and macrophyte cover in Oak Plain Pond. Therefore according to the HSI scores, these ponds have near-ideal conditions for great crested newts and are therefore likely to support healthy populations of this species.

3.5.2. Capture overview

Results from this five year study in four ponds shows high variability in the number of great crested newts captured between ponds and years. Fluctuations in the incidences of capture in great crested newts have been noted in several

studies (Cooke, 1986; Cooke 1995, Cook & Arnold, 2003; Hagström, 1978) and may be linked to changes in detection probabilities resulting from differences in survey conditions (e.g. weather, site conditions). The large fluctuations in counts from this study highlight the need to calculate detection probabilities before making inferences on survival and population size (Bailey et al., 2004b; Baker, 1999). For example, the low numbers of individuals captured from Speakman's Pond may suggest a low population size but probably indicates very low detection probabilities. Two ponds (Oak Plain and Frog) had similar numbers of captures in all years (except 2011) but incidences of recapture fluctuated greatly between sites and years. The low incidence of recapture in Oak Plain Pond may be due to a number of factors including: low annual survival, weak site fidelity, low site propensity, low detection or a combination of these (Sandercock, 2006).

The calculated incidences of recapture in my study were within the range of other studies on amphibians. In a pond in England, Baker (1999) obtained recapture rates of between 22 and 86% in male and 26 to 90% in female great crested newts. In other amphibian species, incidences of recapture in *Bufo boreas* in the USA were within the same range, varying between 51 and 68% (Scherer et al., 2005). In contrast, in a study of gopher frogs *Rana capito* and *R. sevosa,* Richter & Seigel (2002) calculated incidences of recapture at just 13.5% and in the marbled salamander *Ambystoma maculatum*, Gamble et al. (2008) estimated recaptures to be as low as 2 - 3%. Thus it appears detection probabilities vary with species and location, indicating the importance of location- and species- specific studies to determine detection rather than relying on values from just one study or species.

In my study, the observed increase in incidence of recapture between 2007 and 2009 is expected in a stable population as greater numbers of individuals are captured and identified. However a decrease in the number of recaptures occurred in the period 2010 to 2011 which may be due to individuals becoming more trap aware (Schmidt & Anholt, 1999) or an increase in juvenile recruitment to the adult population.

3.5.3. Population survival and detection

The most parsimonious model {Φ(sex), p(time + pond)} as selected in program MARK shows clear support for the data. This model indicates that annual survival remains constant with time and pond but there are differences between males and females. The estimated survival values of 0.40 for males and 0.58 for females is lower than that found in some other studies (Cooke & Arnold, 2003; Hagström, 1979) but within the range of other research (Arntzen & Teunis, 1993; Baker, 1999; Griffiths et al., 2010). In this study there were no differences in survival between ponds. Other research has shown that survival can vary with site (Gill, 1978) but this usually occurs when conditions vary considerably between locations. Ponds in this study were less than 400 m apart and experienced similar environmental conditions. Stable survival among sites suggests that population size is less likely to be driven by variations in adult survival but rather fluctuations in survival of other life stages including eggs, larvae and juveniles (Griffiths, 1997; Griffiths et al., 2010; Williams, 1999). Many studies have reported large fluctuations in annual larval counts (Kupfer & Kneitz, 2000; Miaud et al., 1993). These findings are contrary to those of Schmidt (2005) who found that although survival in the fire salamander *Salamandra salamandra* varied between sites, larval recruitment had little effect

on population processes. Overall it appears that factors governing population processes are location-specific, making it difficult to apply generalisations to population survival estimates.

Annual survival in this study did not vary with time as has been previously reported in some great crested newt populations (Arntzen & Teunis, 1993; Baker, 1999; Cooke & Arnold, 2003; Griffiths et al., 2010; Williams, 1999). The period 2007 to 2010 was characterised by large variations in mean winter temperatures. The winter of 2006 - 2007 was mild (mean minimum 2.9 °C) whereas in 2009 - 2010 and 2010 - 2011 it was exceptionally cold (-1.68 °C and 1.06 °C respectively). December 2010 was reported as the coldest on record (Met Office, 2011). Despite these differences in overwinter temperature no observable change in annual survival was noted in this population. Long term studies on amphibians show a negative effect of mild winters on annual survival in male common toads *Bufo bufo* (Reading, 2007) and great crested newts (Griffiths et al., 2010). This trend was not recorded in this short term study and highlights that long term studies of more than 15 years are required to determine the effects of subtle changes in climate variables, such as temperature and rainfall, on amphibian populations (Salvidio, 2009). It appears that in the short term, this species is able to buffer changes in winter temperature. Effects of climate change may become apparent over many decades, often when interactions occur with other factors such as introduced diseases (Lips et al., 2004).

Annual survival varied with sex with females having higher survival than males, a similar finding to that by Gamble et al. (2009) in marbled salamanders

Ambystoma opacum but contrary to the results of Loman & Madsen (2010) who both found that males had higher survival than females in *B. bufo*. However several studies have reported no differences in survival between the sexes (Anholt et al., 2003; Hagstöm, 1979; Williams, 1999). Therefore it appears that factors operating at specific sites and within species may determine whether differences in survival occur between the sexes. In general, female great crested newts live to a greater age (Verrell & Halliday, 1985), attain larger size (Francillon-Viellot et al., 1990) and have higher survival than males (Gill, 1978; Halliday & Tejedo, 1995). Bailey et al. (2004a) observed reduced survival in males which was attributed to temporary emigration, pond hydrology and pond freezing during winter. A reduction in male apparent survival in this metapopulation may be due to potential temporary emigration, or the effects of breeding or long-term winter conditions. A decline in adult survival over a long time period as observed by Băncillă et al. (2010), Reading (2007) and Griffiths et al. (2010) may be occurring in this population but was not apparent in this short term study. Monitoring over a longer time period will be required to ascertain whether climatic variables are affecting male overwinter survival. If so, this may have negative consequences for future population persistence at this site.

Detection probabilities fluctuated widely between years and sites, a phenomenon noted in other amphibian studies (Canessa et al., 2012; MacKenzie & Kendall, 2002; Schmidt, 2005; Weidong & Swihart, 2004). Frog Pond had the lowest variation (38 to 60%). In Lily Pond detection probabilities fluctuated between 0 and 52%. Oak Plain had the lowest detection probabilities at between 0 and 13%. This variation is likely to be due to differences in

individual behaviour, weather and site conditions (Hyde & Simons, 2001; Kinkead & Otis, 2007). Both Frog and Oak Plain ponds had very different detection probabilities despite being affected by the same weather and having similar habitat conditions, as reflected in HSI scores (0.84 and 0.92 respectively). Both are of a similar age (> 30 years), do not contain fish, contain between 50 and 75% vegetation cover and are relatively undisturbed (although both are used by students in dip netting studies). Oak Plain Pond is just under twice the size of Frog Pond, but despite using double the number of traps detection was still extremely low. Aquatic vegetation in Oak Plain was denser and more widespread than in Frog Pond, perhaps resulting in females dispersing more evenly across the pond and being less available for capture. Otherwise there are few differences between the two ponds. Subtle differences in pond microhabitat conditions may therefore play an important role in detection and may need to be considered in more detail in studies which rely just on count data.

Lily Pond is only 26 m from Frog Pond yet had a larger range in detection probabilities (0 to 52%), with no newts detected in 2011. Lily Pond is extensively used by students for dip netting. The adjacent Field Studies Centre runs approximately 2,000 sessions per year (Field Centre records). This continued disturbance may deter great crested newts from utilising the shore, which was absent of vegetation. Great crested newts generally prefer quieter, more secluded ponds (Cooke & Scorgie, 1983) so may be deterred from utilising this pond. However dip netting levels were consistently high in all years (Field Centre records) and despite clearance in two years, shoreline vegetation abundance did not vary significantly through the study (personal observation) so

these factors are unlikely to account for the variation in detection probabilities. Lily Pond had a lower estimated population size (see below) than the other ponds, so changes in detection due to weather differences may have been the primary cause of fluctuations in detection probabilities. These results again highlight the importance of determining detection probability at every site used, rather than applying generalisations to ponds within a given area.

Estimates for detection could not be calculated in Speakman's Pond due to the extremely low number of captures. This large seasonal pond is surrounded by dense woodland and a series of minor roads. Studies show that roads form barriers to migrating newts (Cushman, 2006; Kupfer & Kneitz, 2000) though traffic density of less than 20 cars per hour is unlikely to be a threat (Oldham et al., 2000). The roads surrounding this pond are classed as minor but traffic densities reach between 100 and 300 cars per hour midweek (personal observation) and rise higher during commuting times, during weekends and evenings due to people visiting a nearby public house. Therefore immigration from other ponds may be limited resulting in low populations and detection. In addition, this pond is dominated by the invasive species *Crassula helmsii* which has narrow leaves. There is evidence to suggest that great crested newts fail to lay eggs on these (Watson, 1994) so this may be preventing egg-laying and further recruitment.

3.5.4. Inter-pond movements

Over the five year period of this study only 11 movements by adults were recorded between ponds. This represents just 0.85% of the 1,289 individuals captured. The low level of movement and high degree of site fidelity in

individuals suggests that populations in this study exist within a metapopulation, rather than as a collection of patchy populations (Hanski & Gyllenburg, 1993). In this area juveniles rather than adults may facilitate genetic exchange between populations. Movements of adults between these ponds may be important in population persistence and maintaining a degree of independence in pond dynamics. In a similar study in Kent, Williams (1999) noted only eight individuals make movements between ponds between years, representing a similarly small proportion of the metapopulation. Other amphibian species may make more numerous movements between patches such as the alpine salamander *Mesotriton alpestris* (Kopecký et al., 2010), the edible frog *Rana esculenta* (Peter, 1991) and the spotted salamander *Ambystoma maculatum* (Purrenhage & Niwiarowski, 2009). These species exist in patchy populations and may have a greater degree of genetic flow of adults between ponds and less independence in pond dynamics.

3.5.5. Population sizes

Estimated population sizes fluctuated greatly between ponds and years which is typical of many amphibian species (Loman & Andersson, 2007; Pellet et al., 2006; Schmidt, 2005). These results demonstrate the importance of long term studies (> 15 years) before deductions are made about population status (Pechmann et al., 1991; Salvidio, 2009). Lily Pond had the lowest (3 to 92 individuals) and Oak Plain the highest (11 to 1,236 individuals) population estimates. Similar fluctuations have been noticed in great crested newt populations in previous studies (Arntzen & Teunis, 1993; Cooke, 1995; Glandt, 1982; Hagström, 1979) indicating that this pattern is not unusual. Arntzen & Teunis (1993) state that population change is governed through early life stages

because larval and juvenile survival is highly variable. Since apparent annual adult survival was constant between ponds in my study, the observed variation in population size is likely to be driven by fluctuations in larval recruitment. Although peak populations in these study ponds were not in the same year, population sizes appear to fluctuate in synchrony with high years followed by low ones. Classic metapopulation theory (Levins, 1969) states that to persist, sub-populations need to fluctuate asynchronously (Baguette, 2004; Hanski, 1998). However this has not been reported in other studies of great crested newt populations (Griffiths et al., 2010; Williams, 1999). In great crested newts, extinction of sub-populations is often deterministic and not stochastic (Smith & Green, 2005), with sub-populations only going extinct if factors such as succession or pond destruction occurs (Hanski, 1998). Whether sub-populations processes are governed by annual survival or larval recruitment, data from my research and those of others indicate that having several ponds in one area is likely to buffer metapopulation extinction.

The maximum population size of the metapopulation in this study was approximately 2,100 individuals in any given year. Minimum viable population size (MVP) can be defined as the smallest size required for a population or species to have predetermined probability of persistence for a given length of time (Shaffer, 1981). Reed et al. (2003) proposed that a population must exceed 5,800 individuals for 95% likelihood of persistence over 40 generations. However Halley et al. (1996) suggest that a population of only 100 individuals is required. Di Minin & Griffiths (2011) state that population viability analysis must be population-specific as well as species-specific. Therefore an estimated minimum viable population size from one study is unlikely to hold true at all

sites. Populations of great crested newts have been present at this study site for over three decades (Pickett, 1986). The viability of the population in the future will depend on annual survival and recruitment along with sufficient movements between ponds to maintain genetic flow and to rescue declining populations.

3.5.6. Sex ratio

The observed sex ratio varied between ponds and years. Lily Pond was alternately dominated by males then females. The reason for this observation may be due to the low population size and resulting biases in calculating accurate sex ratio. Similarly, any variation in the sex ratio in Speakman's Pond is likely to be due to the low number of captures. Both Frog and Oak Plain ponds had high captures but largely different sex ratios. Frog Pond was dominated by females in every year except 2007, at a sex ratio of approximately 2:1. In contrast, Oak Plain was dominated by males from 2007 to 2009 and females from 2010 to 2012. The observed or operational sex ratio (Sullivan et al., 1995) can be affected by differences in the amount of time sexes spend at the pond and are thus available for capture (Latham & Oldham, 1996), temporary emigration (Muths et al., 2006; Willson et al., 2011), permanent emigration (Schmidt et al., 2007) or differences in annual survival (Wood et al., 1998). In this metapopulation detection probabilities were similar for both sexes, indicating that variation in the sex ratio were likely to be the result of different numbers of each sex present at breeding ponds rather than observer error. In general males spend more time at breeding ponds than females (Latham & Oldham, 1996), which often leads to a male biased operational sex ratio (Loman & Madsen, 2010). However in my study population females appeared more prevalent than males. This may be due to differences in the emigration

rate, or levels of survival between the sexes. Temporary emigration may be more prevalent in males than females (Bailey et al., 2004a) but this behaviour was not identified in my study. Permanent emigration may also have occurred but my findings suggest very high site fidelity, so this behaviour is unlikely to contribute significantly to the observed sex ratio. Annual survival in males was estimated to be lower than females. This could have led to a female dominated population in Frog and Oak Plain ponds (in later years).

3.5.7. Critique of methods

1. Temporary emigration was not estimated which may have impacted on estimates of apparent survival and detection. This behaviour has been noted in some amphibian species (Bailey et al., 2004a, 2004b; Schmidt, 2005) but not others (Pellet et al., 2007; Schmidt et al., 2007) and more advanced techniques such as Pollock's robust design (Pollock, 1982) would have identified whether this behaviour was taking place. However this analysis was beyond the scope of this study. Further time and resources for analysis may make this possible in the future.

2. Low detection from Oak Plain and Speakman's ponds led to wide confidence levels in estimates for apparent survival. More intensive trapping may have helped solve this issue but due to time and resource constraints this was not possible.

3. Inter-pond movements may have been higher than those observed but due to the low incidences of recapture, this was difficult to determine accurately. More intensive trapping may have solved this issue.

3.6. Conclusions and implications for conservation

1. Incidences of capture fluctuated widely between seasons and ponds. However this was not reflected in the actual population size estimates between ponds and highlights the importance of calculating detection probabilities before inferences on population status are ascertained (Bailey et al., 2004d). Surveys carried out on one or two nights are likely to give little information as to the population status of the pond due to variation in detection probabilities.

2. Pre-mitigation surveys are often carried out to determine the presence and population status of great crested newts. At present surveys rely on count data to make estimates of population size (Griffiths & Inns, 2003). Results from this study demonstrate that failure to estimate detection probabilities will result in biased estimates of population status. Therefore pre-mitigation studies should aim to make estimates of detection by the use of capture-recapture techniques. This will provide more accurate estimates of population sizes for potential protection or translocation.

3. Annual survival varied by sex but not season or site. This demonstrates the importance of calculating population-specific values for apparent survival since species-specific information may vary with sites and years. In this metapopulation females had higher survival than males. Conservation measures should aim to promote annual survival in both sexes, particularly males, by ensuring breeding and terrestrial habitats are kept optimum for this species. This will involve continued use of the HSI score on ponds to determine

any changes in their suitability. Terrestrial habitat should also be monitored to ensure there is no loss or degradation in this area.

4. Only 11 inter-pond movements were observed in this study. This indicates that where conditions are suitable great crested newts may not move between ponds very frequently. This movement may be enough to maintain metapopulation persistence by the rescue effect from source ponds but may increase the time taken for new populations to become established. Therefore management should aim to ensure each pond is maintained and suitable for great crested newts to limit any extinction of any populations.

5. Population sizes were significantly different between ponds and years, with little link between incidences of capture and actual population size. This highlights the need for studies of several years using the capture-mark-recapture approach before inferences on numbers of individuals can be made. At this particular site, Oak Plain had the largest population so this pond should be monitored to ensure no significant habitat changes occur which may detrimentally affect this species.

Chapter 4. Ecology of juvenile great crested newts *Triturus cristatus* in their terrestrial phase.

4.1. Abstract

This study examined the terrestrial ecology of juvenile great crested newts *Triturus cristatus* from March to October over four years. Patterns and incidence of capture varied considerably with refuge type. Juveniles were significantly more likely to be encountered under concrete refuges compared to natural logs or large stones. Six cover objects provided consistent captures indicating that juveniles may remain within small home ranges close to natal ponds to feed and grow for many months. Numbers of captures were significantly correlated with mean monthly minimum air temperature but not rainfall. Juvenile growth rates were significantly higher in the size class (SVL) 35 to 39 mm compared to the four categories encompassing SVL's of 40 to 59 mm. Body condition indices (BCI) varied significantly with season, with the highest values in spring and lowest in summer. BCI scores were highest after milder winters although there was no significant relationship between spring BCI scores and mean winter air temperature. There was no significant relationship between individual body condition and growth rate. Apparent monthly survival was constant in three years at between 0.56 and 0.90. Detection probabilities for the six refuges were constant at between 0.69 and 0.89. Estimated juvenile apparent annual survival was constant at 0.19 while population size fluctuated between years.

4.2. Introduction

Many species of pond-breeding amphibian spend the majority of their lives on land (Beebee, 1977a). Despite this, our understanding of amphibian behaviour and ecology during their terrestrial phase remains poorly understood (Malmgren, 2007; Oldham et al., 2000; Regosin et al., 2003) and lags far behind that of the aquatic phase (Schabetsberger et al., 2004). This is mainly due to the secretive behaviour of amphibians when on land (Malmgren, 2007). In recent years studies have used a variety of tracking devices to follow adult amphibians once they leave breeding ponds (Forester et al., 2006; Jehle & Arntzen, 2000; Miaud et al., 2000; Montieth & Paton, 2006) and these have given useful insights into emigration direction (Jehle & Arntzen, 2000), distances travelled (Bonato & Fracasso, 2003; Muths, 2003) and the range of microhabitats utilised (Jehle & Arntzen, 2000; Schabetsberger et al., 2004). These studies have revealed that great crested newts leave aquatic habitats at the end of the breeding season, which in great crested newts may be between June and October. Adults tend to emigrate towards favoured habitats such as woodland, scrub and bare soil (Schabetsberger et al., 2004). Deciduous woodland and rank grassland are favoured habitats (Latham & Oldham, 1996) but adults, in particular, orient towards patches of bare ground (Jehle & Arntzen, 2000) suggesting that underground refuges are particularly important. The Italian crested newt (*T. marmoratus*) utilises refuges including: burrows of rodents, cavities under rocks and rotting tree stumps from depths 5 to 80 cm (Schabetsberger et al., 2004). During periods of cold weather, including frost, individuals appear to undergo vertical migration into the soil (Watson, 1994). The length of time that individuals spend on land appears to vary with species

and location but typically adults will remain in terrestrial habitats until temperatures rise the following spring.

Terrestrial cover objects are commonly found close to ponds, especially in deciduous woodland habitats. These usually take the form of dead logs and stones and are often utilised by adult amphibians whilst in their terrestrial phase (Marnell, 1998), which in temperate species lasts from about August/September to February/March. Natural cover objects such as logs and stones or artificial objects such as pieces of old carpet that are utilised by terrestrial amphibians enable more effective studies (Hampton, 2007; Marsh & Goicochea, 2003; Smith & Petranka, 2000). The advantages of using artificial cover objects include: low time commitment, lack of habitat destruction, repeatability at the same station, relative safety to animals and standardisation of the number and area of cover items (Monti et al., 2000). If examined at regular intervals animals can be found on a more regular basis and population studies are more effective. Research on smooth newts using such an approach has shown that adults show high fidelity to particular refuges and inhabited areas around ponds (Griffiths, 1984).

Metamorph and juvenile amphibians are particularly difficult to follow whilst on land due to their small size and extremely elusive nature. Attaching radio tracking devices is therefore difficult (Vos et al., 2007) and so there are relatively few studies which have examined juvenile terrestrial behaviour. Consequently, there is a lack of knowledge of juvenile compared to adult ecology. The limited research that has been undertaken indicates that juveniles may be more vagile than adults and often appear to colonise new pond habitats

(Breden, 1987; Sinsch, 1997). However some studies seem to indicate that high variability occurs in the degree of juvenile movement in different species (Timm et al., 2007). For example, juvenile pool frogs *Pelophylax lessonae* may roam several hundred metres from natal ponds in a few days (Sinsch, 1997) while great crested newt metamorphs may emigrate up to 800 m in one year (Kupfer & Kneitz, 2000). When leaving ponds newt metamorphs may emigrate towards favourable habitats (Hayward, 2010), with some evidence suggesting they may follow chemical cues left by adults (Hayward et al., 2000). Once on land it appears that juveniles utilise similar types of microhabitat to adults including under logs, in leaf litter and in soil (Vos et al., 2007). On occasion it appears that juveniles may return to water to feed (Verrell & Halliday, 1985). However this may only occur when foraging conditions in terrestrial habitats are relatively poor (Verrell & Halliday, 1985). Overall, these studies demonstrate that extent to which juveniles of different species vary in their terrestrial behaviour but no research has been conducted to examine how juvenile great crested newts utilise terrestrial cover objects in a deciduous woodland habitat.

Many studies have examined adult amphibian growth rates during both aquatic and terrestrial phases (e.g. Arntzen, 2000; Francillon-Viellot et al., 1990; Marvin, 2001; Sever et al., 2001). Growth rates in amphibians appear to be highly variable among individuals, species and years (Ash et al., 2003; Gibbons & McCarty, 1984) and in most aquatic-breeding species size is a poor indicator of age (Halliday & Verrell, 1988; Sullivan & Fernandez, 1999). Many amphibians appear to exhibit rapid growth up to the first year of reproduction, after which growth rate declines (Marvin, 2001; Sever et al., 2001). Therefore large adult size may indicate faster juvenile growth rate (Halliday & Tejedo, 1995) rather

than an older individual. Males usually grow faster than females and may reach reproductive maturity earlier (Castanet et al., 1996; Khonsue et al., 2001). Growth appears to vary through the year with slower rates in times of extreme hot or cold. For example, adult southern crested newts *Triturus karelinii* aestivate in hot summers (Olgun et al., 2005), while juvenile marbled newts *Triturus marmoratus* hibernate in the winter (Jakob et al., 2002), both of which results in slower growth. Research on growth rates in amphibians has generally concentrated on adults with relatively few studies on juveniles. However a study by Arntzen (2000) on juvenile great crested newts revealed that growth rates varied with season and year with growth being fastest in the first three years. Arntzen & Teunis (1993) found juvenile growth to be fastest in the summer months. Given the paucity of data in the area of juvenile ecology, further research is required to determine growth rates of juvenile great crested newts in different habitats and under different environmental conditions.

Amphibian population research is usually carried out at breeding ponds (e.g. Baker, 1999; Blackwell et al., 2004; Hels & Nachman, 2002; Williams, 1999) and due to their secretive behaviour and low detection (Muths et al., 2006), estimates of apparent survival, detection probability and population sizes of juvenile amphibians whilst in their terrestrial phase are rarely conducted. Estimating these parameters is crucial in understanding amphibian population dynamics and for implementing conservation measures (Bailey et al., 2004a; Di Minin & Griffiths, 2011; Schmidt et al., 2004). For example, low juvenile survivorship may result in low recruitment to the adult population and have implications for long-term population persistence.

Body condition indices give an indication of the relative health of an individual and may be useful in showing the current status of a population and quality of individuals (Janin et al., 2011). Studies examining body condition in adult amphibians are widespread (e.g. Baker, 1992; Cooke & Arnold, 2003; Kopecký et al., 2010; Lowe et al., 2006) and generally suggest that amphibians have a higher body condition at the start of the breeding season compared to the end (Arntzen et al., 1999). This is due to the costs involved in reproduction (Arntzen et al., 1999). Research examining the body condition index of metamorphs and juveniles are relatively rare. However an understanding of how individual body condition changes temporally and the abiotic factors which affect body condition are important for understanding population survival. There is some evidence to suggest that factors such as temperature at breeding ponds affect metamorph body condition (Reading, 2010), but no studies have demonstrated links with body condition in the period after leaving ponds and before first breeding.

The aims of this study were to:

- Examine the extent and use of terrestrial cover objects in juvenile great crested newts in a deciduous woodland area.
- Determine the snout-vent length (SVL) growth rates of terrestrial juveniles in the period March to October over four consecutive years.
- Calculate body condition index (BCI) of juveniles over four years.
- Use an information-theoretic approach in program MARK to estimate apparent monthly and annual survival and detection in juveniles.
- Estimate population sizes of terrestrial juvenile great crested newts in an area of deciduous woodland over a four year period.

4.3. Methods

4.3.1. Location of terrestrial refuges

A total of 63 refuges were placed in a three-hectare area surrounding Epping Forest Field Centre (Figure 4.1). All refuges were placed on natural substrate, usually leaf litter, soil or grass, in small rings or lines of between three and 8 to allow ease of identification and sampling (see Appendix 2. for details of refuge size and arrangement). Forty-eight refuges were natural logs of varying size. Six refuges were flattened stones, while the remainder consisted of 9 concrete slabs. Length, diameter and circumference (± 1.0 cm) of logs and stones was measured to enable calculation of refuge volume. The length, width and depth of concrete slabs were measured instead due to their cuboid shape. Habitats surrounding refuges consisted of grassland, deciduous woodland or dense scrub, all favoured areas for great crested newts (Jehle & Arntzen, 2000).

4.3.2. Sampling methodology

Once a week refuges were carefully lifted to determine the presence of great crested newts. Care was taken not to sample more frequently as this may have affected the newts' behaviour and site fidelity (Marsh & Goicochea, 2003). Sampling was conducted between 10:00 and 12:00 h. Any newts encountered were placed in individual plastic tubs. Adults were identified using body colour pattern markings and residual secondary sex characters. Juvenile palmate and smooth newts are difficult tell apart but were identified by the extent of dorsal stripe (Roberts & Griffiths, 1992). Palmate and smooth newts were immediately returned to the refuge of initial capture.

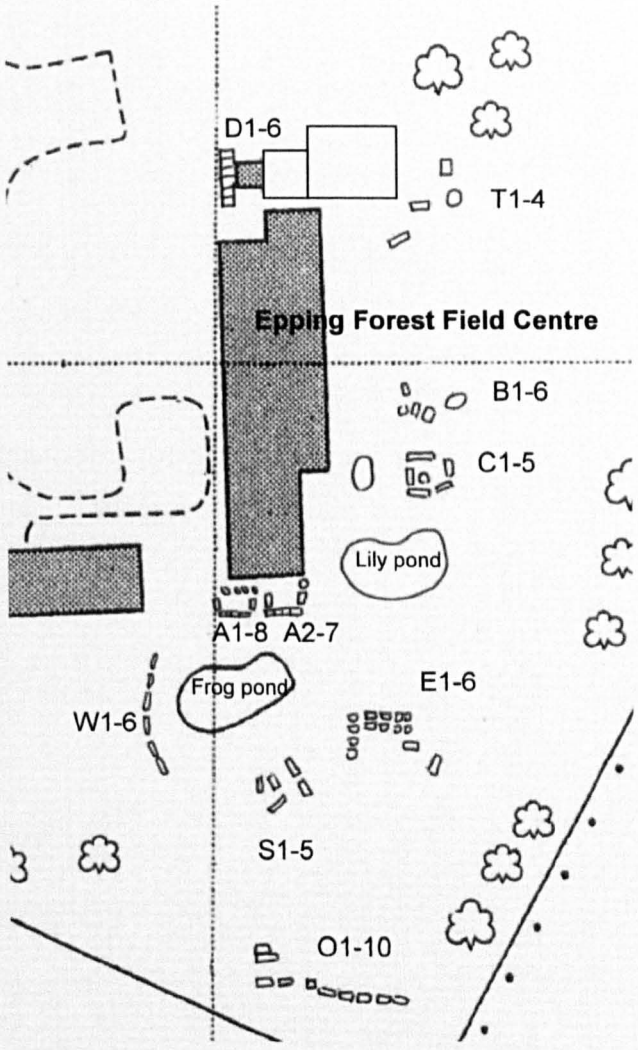


Figure 4.1. Location map of terrestrial refuges situated around the grounds of Epping Forest Field Centre. Locations D1 - 6, E3 & 4 and T3 are concrete refuges; refuges B1 - 6 are stones, the remaining are all natural logs.

Snout-vent length (SVL) was measured for each great crested newt, taken as the distance from the tip of the snout to the posterior side of the cloaca to the nearest 0.5 mm using callipers. Individuals were classed as juveniles if they had a SVL in the range 36 to 59 mm. Fifty-nine millimetres is slightly larger than that used by Dolmen (1983) (55 mm) and Verrell & Halliday (1985) (51 mm). This is because fully-grown adults appeared under refuges rarely and these were clearly identified by either the presence of a reduced crest (male) or large SVL

of 60 to 70 mm (female). Juveniles with a SVL < 40 mm and with the presence of gill slits on the head were classed as metamorphs, i.e. individuals which had recently metamorphosed from larvae. These were grouped with juveniles for data analysis. Body mass was recorded to the nearest 0.01 g using digital pocket scales. Each individual great crested newt has a unique belly pattern marking (Arntzen et al., 1999) which was recorded to allow individual newt recognition (Griffiths, 2001). Digital photographs were taken from underneath by placing newts on clear plastic and covering with a sponge. Individuals were then gently placed back next to the refuge of capture and allowed to crawl back underneath. This avoided accidental crushing of juveniles under refuges.

4.3.3. Data analysis

Single factor analysis of variance (ANOVA) was performed to determine whether there was any difference in: i) the size of refuges of different types (logs, stones, concrete) and ii) the number of juveniles found under different refuge types. Pearson's correlation coefficient was used to determine if there was any correlation between refuge size and juvenile number. Multiple regression analysis was used to determine whether the mean number of juveniles captured was affected by mean monthly minimum air temperature and total rainfall.

Monthly growth rates were calculated for each newt using the formula:

$$\text{Growth rate} = (L_e - L_s) / L_e$$

Where L_e is the SVL at the end of the month, L_s is the SVL at the start of the month. Data from individuals were pooled to give mean growth rates for each month from March to September and for each year. Growth rates were not

recorded during the late autumn and winter months (October to February) due to difficulties in finding terrestrial juveniles. Single factor ANOVA was performed on data to determine differences in growth rate between month and year. Multiple regression was used to analyse the effect of: (i) mean seasonal temperature; (ii) seasonal rainfall on growth rates. Mean seasonal temperature was calculated as the mean minimum daily air temperature for each season, where spring = March to May, summer = June to August and autumn = September to November. Seasonal rainfall was calculated as the total rainfall recorded in each season.

The body condition index (BCI) of each juvenile was calculated using the residuals method. This uses the residuals (y) from an ordinary least squares regression of mass against SVL after log transformation:

$$BCI = (Log_{10} SVL) / (Log_{10} mass)^{y}$$

Juveniles ranged in SVL from 38 to 59 mm and they were divided into age classes prior to body condition analysis. This is because large and small individuals distribute resources differently (Peig & Green, 2010). The proportion of stored energy therefore changes depending on the size of the animal so scaling is required to make meaningful comparisons (Peig & Green, 2010). Single factor ANOVA was performed on data to determine differences in BCI between seasons and years. Linear regression was used to analyse the effect of BCI and: (i) mean air minimum temperature; (ii) total non-aquatic rainfall (i.e. the total amount of rain falling whilst most juveniles are on land) on mean BCI of juveniles in spring of each year; (iii) determine whether BCI affected growth rates of individuals. Mean air temperature was taken as the mean minimum air temperature across the winter period, from December to February each year.

Total non-aquatic rainfall was calculated as the total rainfall in the preceding August to February each year.

Apparent monthly and annual survival, detection and estimated population sizes were calculated for each year using program MARK, as described in Chapter 3. Juveniles from six concrete refuges were only used in the analysis since incidence of recapture from the remaining refuges was nearly zero. This would have led to highly unreliable and inaccurate estimates of apparent survival and detection.

4.4. Results

4.4.1. Patterns and incidence of capture 2008 - 2011

A total of 149 individual juvenile great crested newts were captured 556 times in the period March 2008 to June 2011. There was a significant difference in the size of refuges ($F_{9,62}$ = 8.84, $p < 0.001$). However there was no significant correlation in the number of individual juveniles captured and refuge size ($R = -0.38$, $p > 0.10$. This indicates that juveniles were no more likely to be encountered under smaller compared to larger cover objects.

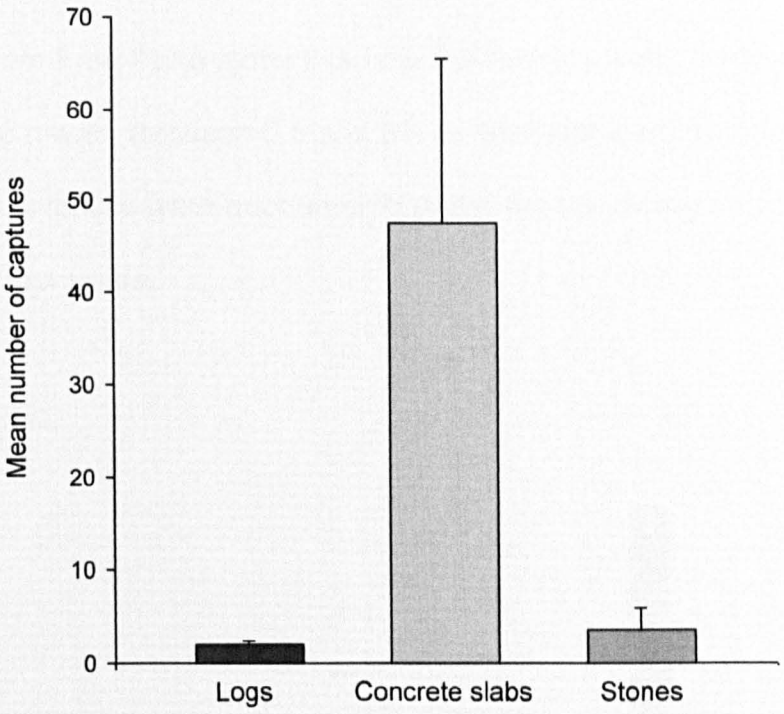


Figure 4.2. The mean number of juvenile great crested newts found under different types of refuge, March to October, 2008 - 2011. Error bars denote standard error.

There was a highly significant difference in the number of juveniles encountered under logs and stones compared to concrete slabs (Figure 4.2) ($F_{2,62}$ = 19.68, $p < 0.001$). Seventy-eight percent of captures were found underneath 8 concrete refuges (locations D & E) with juveniles from location D being recorded in nearly every sampling week. Only 16% and 5% of juveniles were encountered under large stones and logs respectively. This indicates that juveniles showed a strong preference for microhabitats under concrete cover objects compared to stones or logs.

Thirteen percent of captures were in locations A1 and A2 (Figure 4.3). Most of these occurred in September and October. Individuals were in the size range 36

to 39 mm and were identified as metamorphs. These are likely to have emigrated from Frog Pond since this was the nearest water body with breeding great crested newts. Between 0.5 and 5% of captures were from locations B, E, O and T. No juveniles were encountered under the remaining refuges S, W, and C across all four years.

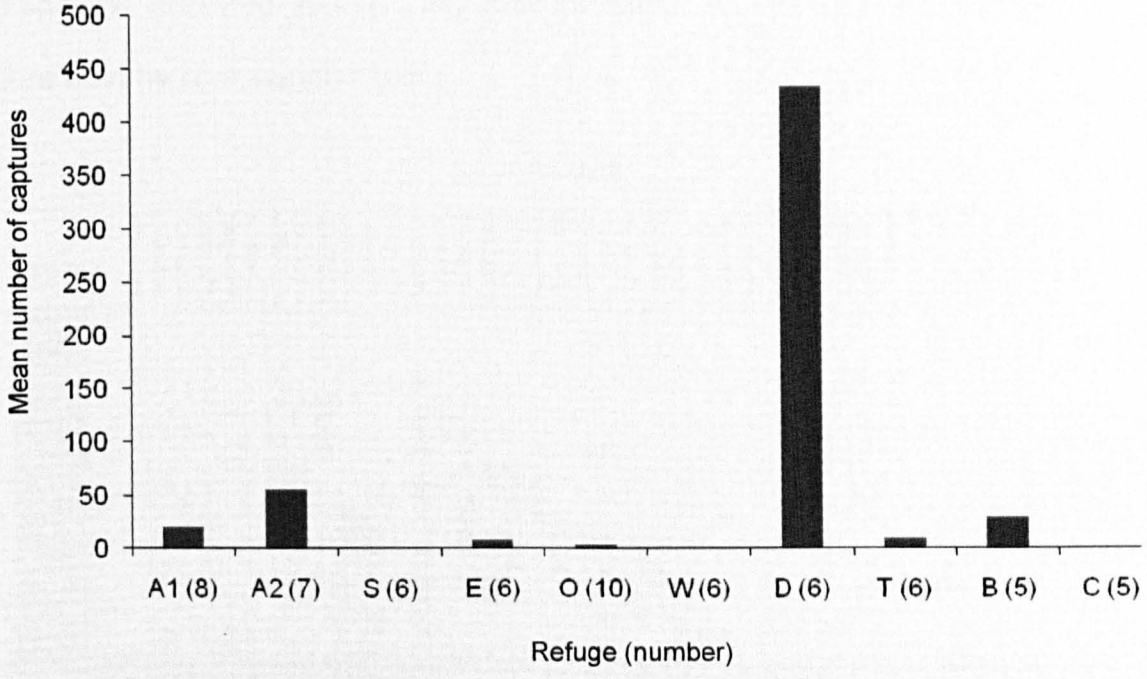


Figure 4.3. Number of captures of juvenile great crested newts under refuges, 2008 - 2011. Letters denote refuge group and the number in brackets refers to the number of refuges within each group.

Forty-two (28%) individuals were captured once. The majority of these were metamorphs located under refuges surrounding Frog pond (locations A1 & A2). After initial capture, these individuals may have undergone permanent or temporary emigration, remained present but undetected or died. The remaining 72% of individuals were captured between two and 19 times, often on consecutive sampling occasions. Fifty-seven (38%) juveniles were captured for

several consecutive weeks, followed by a period of non-detection. This was followed by another period of captures. Individuals would often return to the same refuge and remain there for many months (Figure 4.4). During the winter months (December to February) individuals would disappear from refuges, often re-appearing under the same slab the next spring. Twenty-four individuals (16%) were observed to overwinter and reappear under the same or adjacent refuge the following spring. Only one individual (0.7%) was encountered for more than two consecutive years.

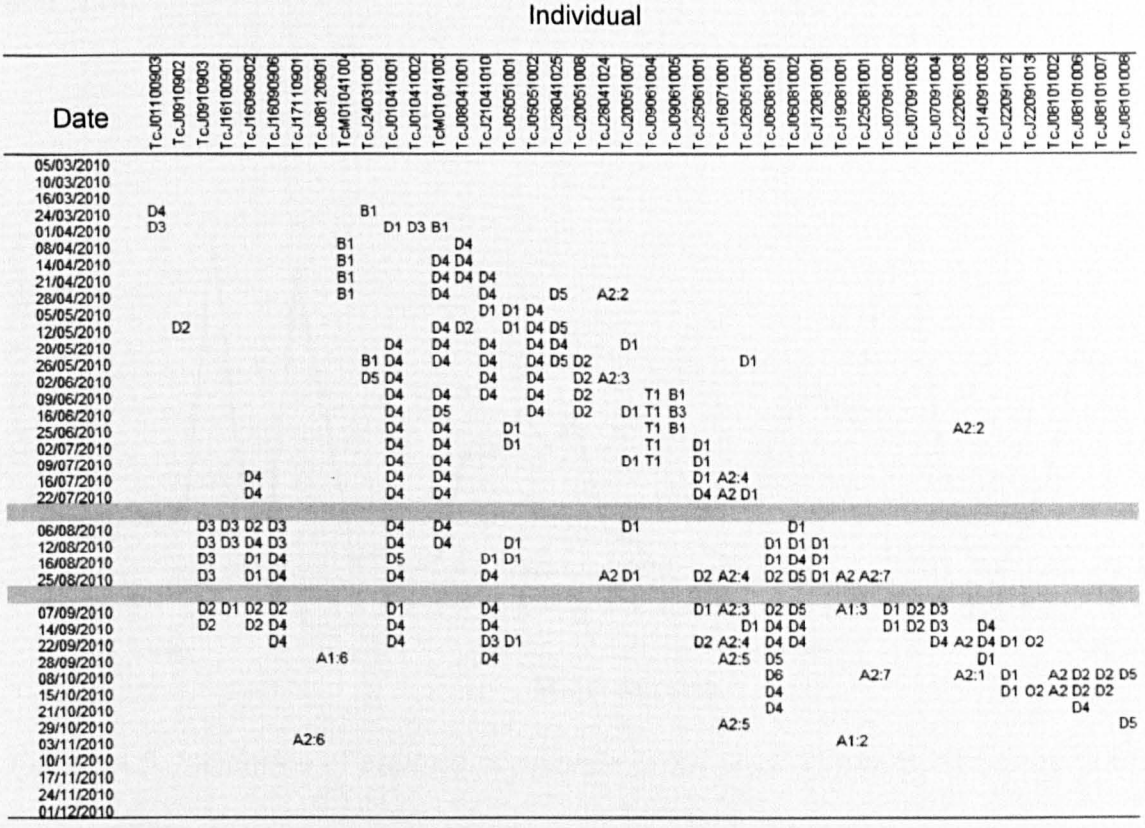


Figure 4.4. Pattern of captures and recaptures for all individual juvenile great crested newts in 2010. Letter/number combinations indicate specific refuge of capture. Grey shading denotes weeks of no sampling.

Patterns of capture varied with season and year (Figure 4.5). Captures were zero in the period December to February. In each year numbers rose through

the spring (March to April) to peak in the summer (June and July) and early autumn before rapidly dropping off in late November. In 2008 there was a bimodal peak in abundance, the first of which occurred in June with 48 individuals and then a second in late September of 42 individuals. Captures in 2009 were lower than the previous year by almost 50% with a peak of 23 juveniles in June. Numbers in 2010 showed a different pattern with a low peak in June of 26 followed by a high peak of 45 individuals in August. Lastly, numbers in 2011 showed a similar start to the year but numbers tapered in May and June.

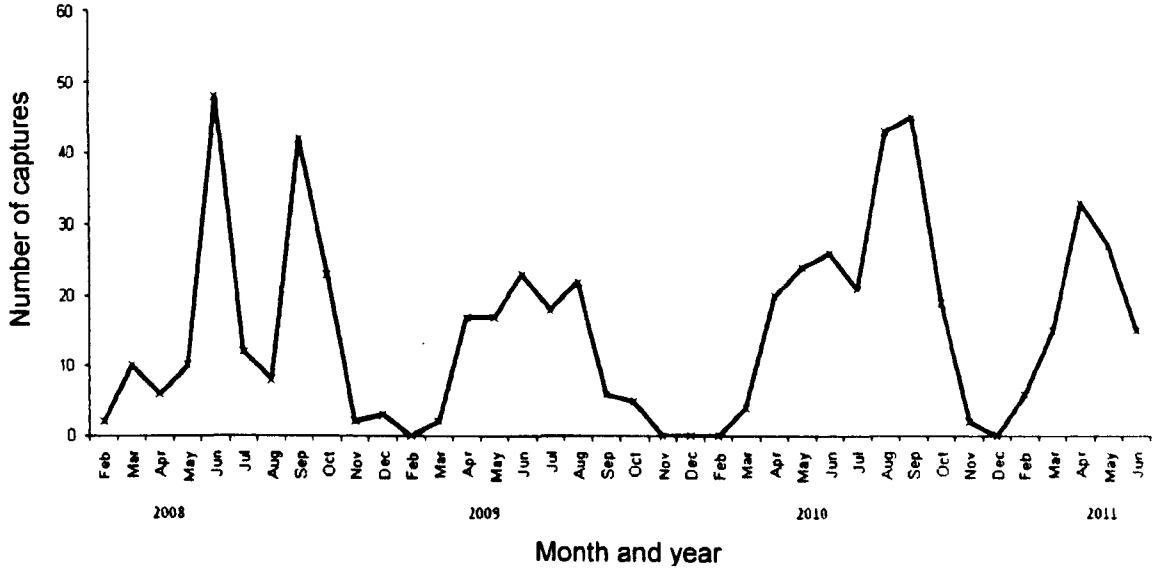


Figure 4.5. Incidence of capture of juvenile great crested newts, February 2008 to June 2011.

Patterns of capture followed mean monthly minimum air temperature (Figure 4.6), which rose through each spring (March to May), to peak in June and July before declining in autumn (late September to November).

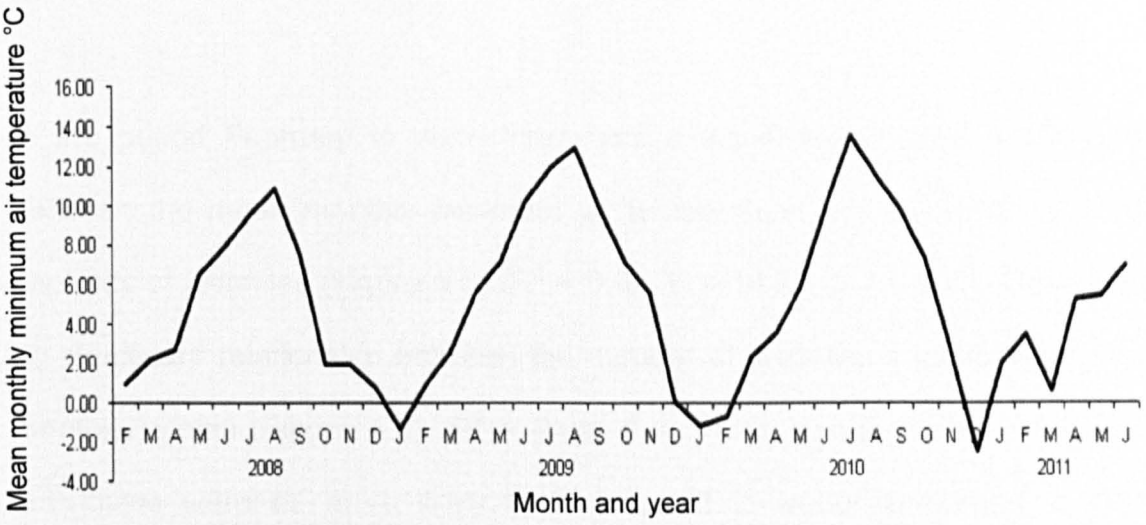


Figure 4.6. Variation in mean monthly minimum air temperature, February 2008 to June 2011.

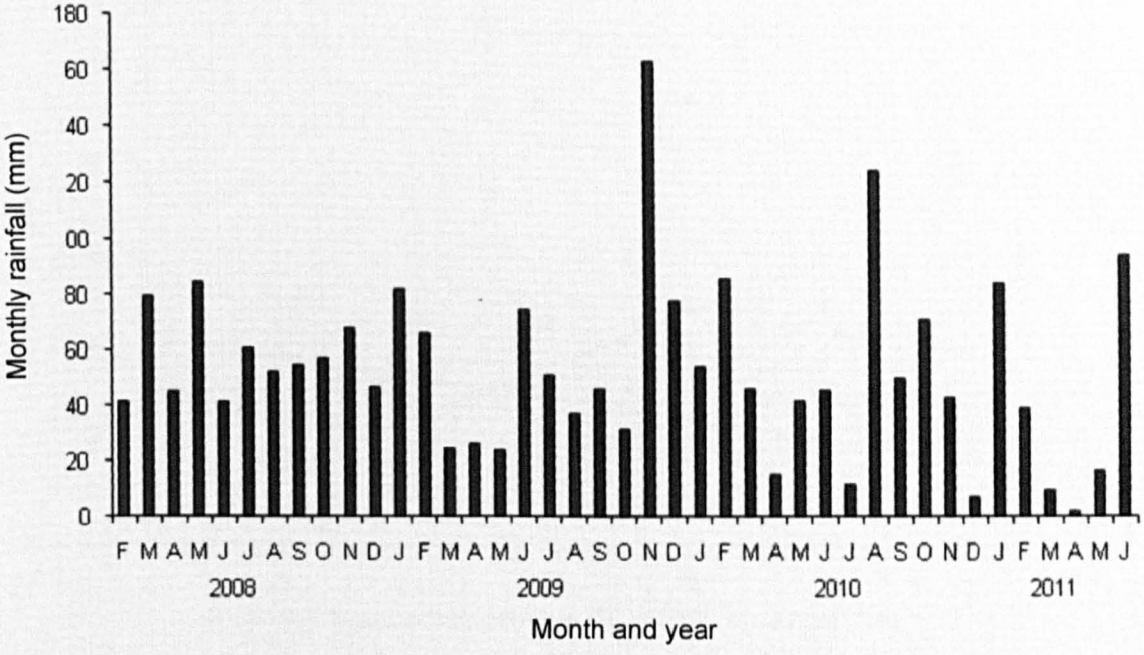


Figure 4.7. Monthly rainfall totals for the period February 2008 to June 2011.

Patterns of rainfall showed considerable variation between months and years (Figure 4.7) with little discernable pattern within each year. Spring 2009 was remarkably dry and very wet in November. In contrast summer 2010 was wetter than average but dry in November.

In the period February to June there was a significant positive relationship between the mean monthly minimum air temperature and the mean monthly captures of juveniles (Figure 4.8) (R^2 = 0.62, F = 14.27, p < 0.001). There was no significant relationship between the number of juveniles captured and total monthly rainfall (Figure 4.8) (R^2 = 0.62, F = 14.27, p = 0.14) indicating that individuals were no more likely to be present in wetter compared to drier conditions.

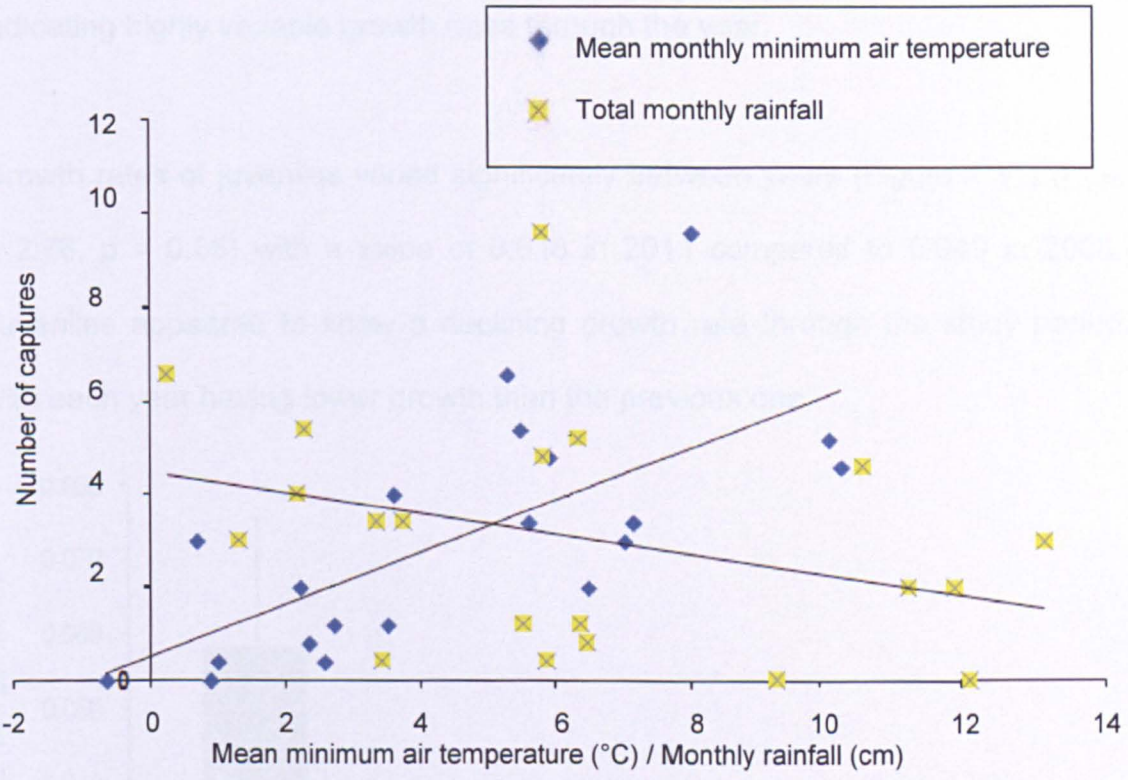


Figure 4.8. Multiple regression to show the effect of mean monthly minimum air temperature and total monthly rainfall on number of juveniles captured.

4.4.2. Growth rate

Mean growth rates in the smallest size class (35 - 39 mm) were significantly faster than those in all remaining size classes (40 - 59 mm) ($F_{1,54}$ = 7.74, p =

0.007). Post hoc analysis revealed that there was no significant difference in the growth rate between the remaining four size categories 40 - 59 mm ($F_{3,46}$ = 0.02, p = 0.99). Mean growth rate fluctuated between 0.022 and 0.031 in age classes 44 - 59 mm (Figure 4.9).

Mean growth rate of juvenile great crested newts varied across the year with the lowest recorded in March (0.014) and April (0.009) and highest during August (0.047) and September (0.034) (Figure 4.10). However these differences were not significant ($F_{6,59}$ = 0.156, p = 1.78) with high standard error for each month indicating highly variable growth rates through the year.

Growth rates of juveniles varied significantly between years (Figure 4.11) ($F_{3,60}$ = 2.76, p = 0.05) with a value of 0.018 in 2011 compared to 0.049 in 2008. Juveniles appeared to show a declining growth rate through the study period with each year having lower growth than the previous one.

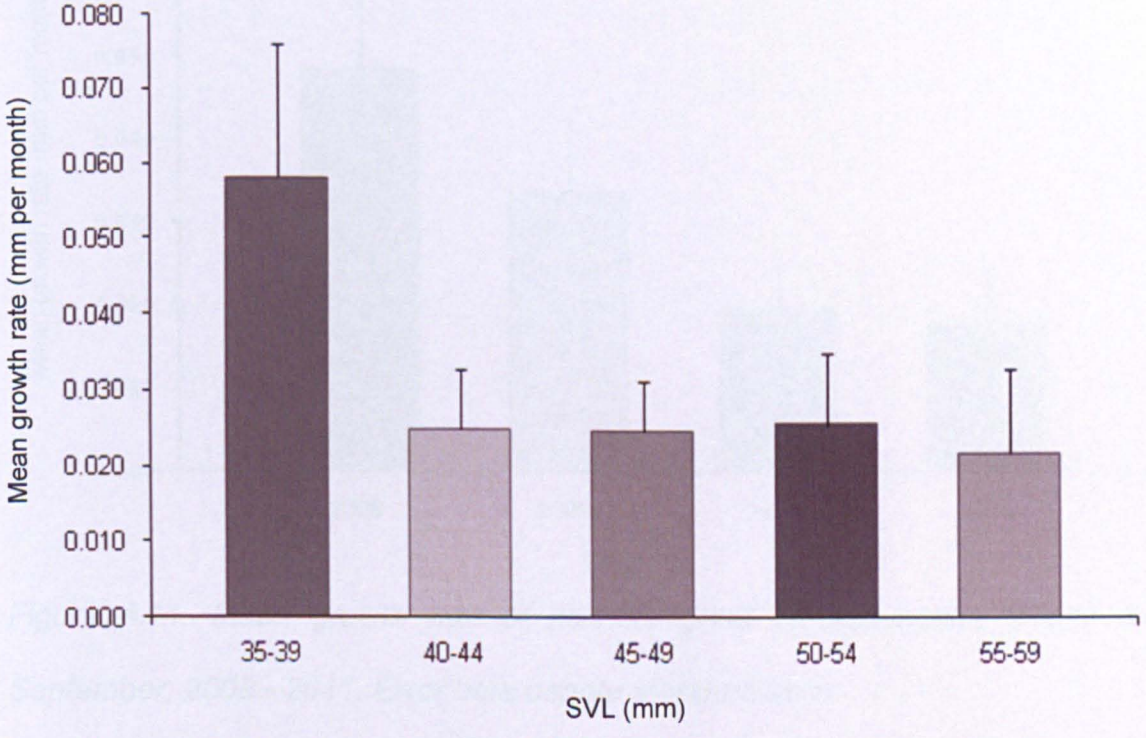


Figure 4.9. Mean growth rate of different juvenile size classes. Growth in size class 35-39 is significantly higher than growth rates than remaining sizes: $F_{1,54}$ = 7.74, p = 0.007. Error bars denote standard error.

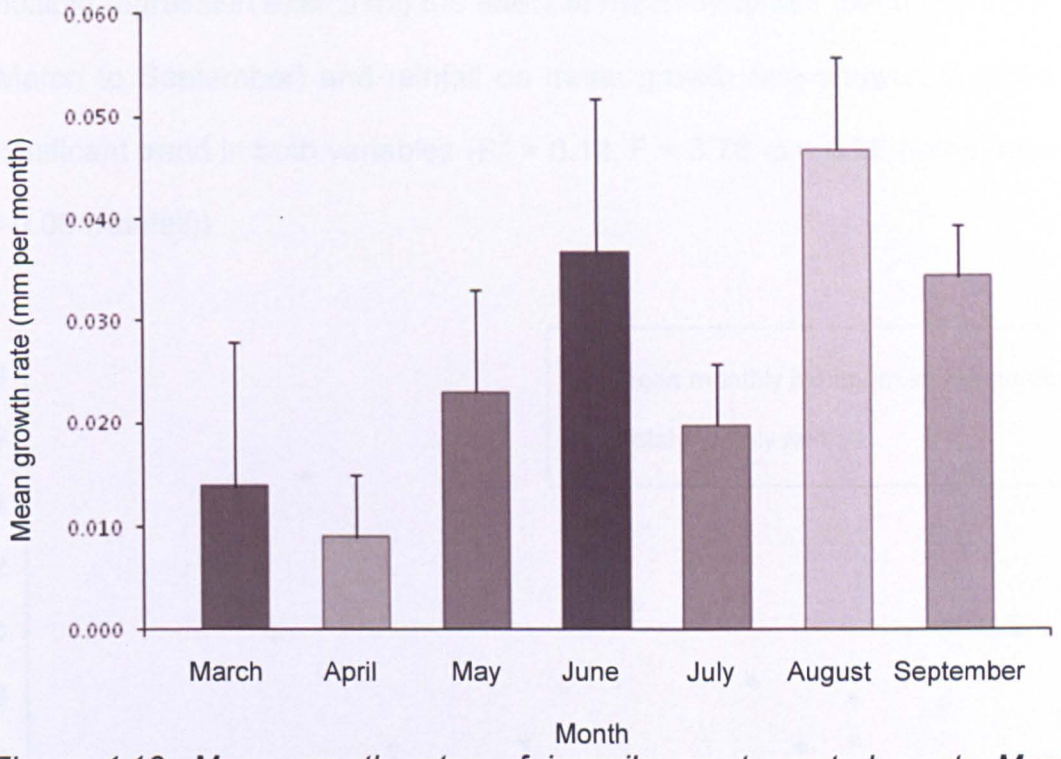


Figure 4.10. Mean growth rates of juvenile great crested newts March to September in the period 2008 to 2011. Error bars denote standard error.

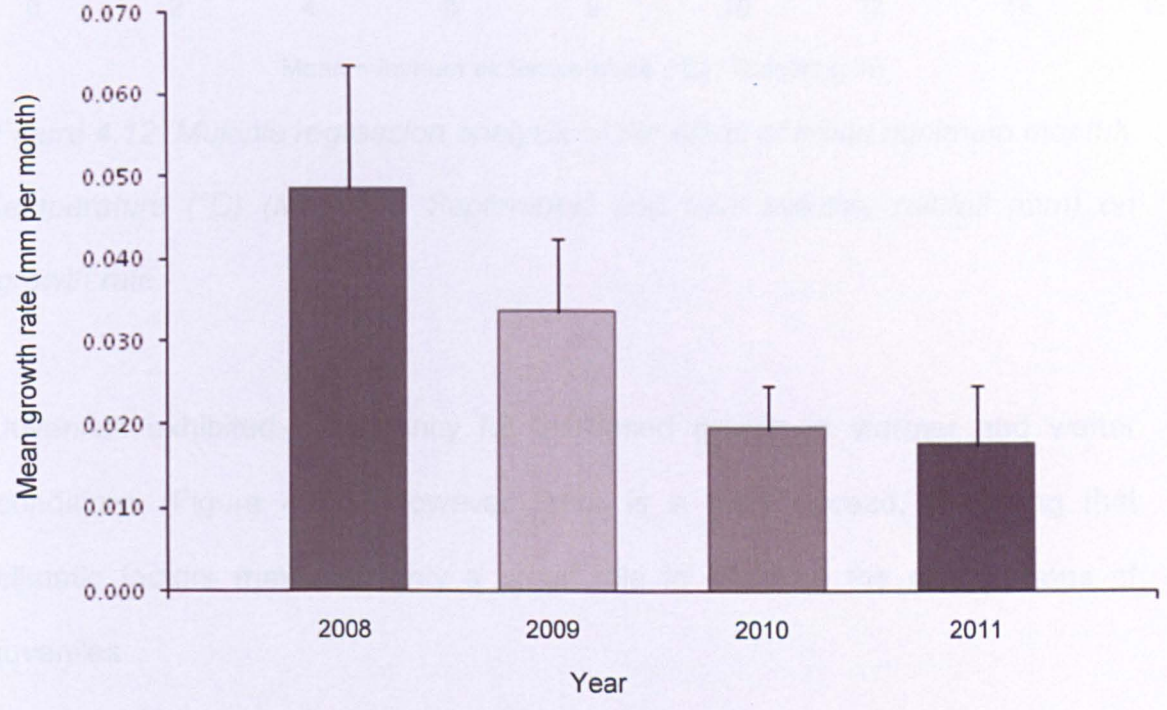


Figure 4.11. Mean growth rate of juvenile great crested newts, March to September, 2008 - 2011. Error bars denote standard error.

Multiple regression examining the effect of mean minimum monthly temperature (March to September) and rainfall on mean growth rate showed a slight non-significant trend in both variables (R^2 = 0.12, F = 3.78, p = 0.25 (temperature); p = 0.06 (rainfall)).

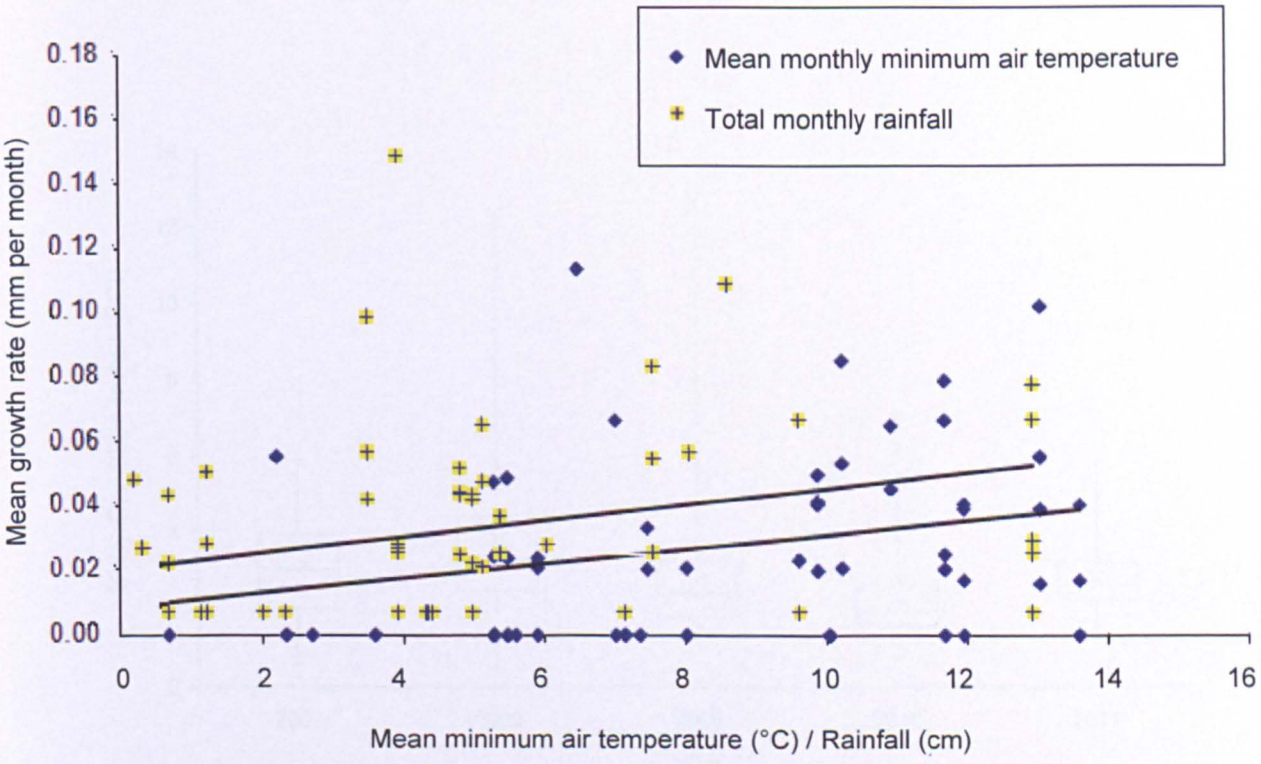


Figure 4.12. Multiple regression analysis of the effect of mean minimum monthly temperature (°C) (March to September) and total monthly rainfall (mm) on growth rate.

Juveniles exhibited a tendency for increased growth in warmer and wetter conditions (Figure 4.12). However there is a large spread, indicating that climatic factors may play only a small role in affecting the growth rates of juveniles.

4.4.3. Body condition

There were significant differences in juvenile body condition index between years (Figure 4.13) ($F_{3,70}$ = 2.84, p = 0.044). Values were highest in 2008 at 4.29 before falling in 2009 and again in 2010 to a low of 2.17. Body condition index rose slightly at the start of 2011 to 2.78.

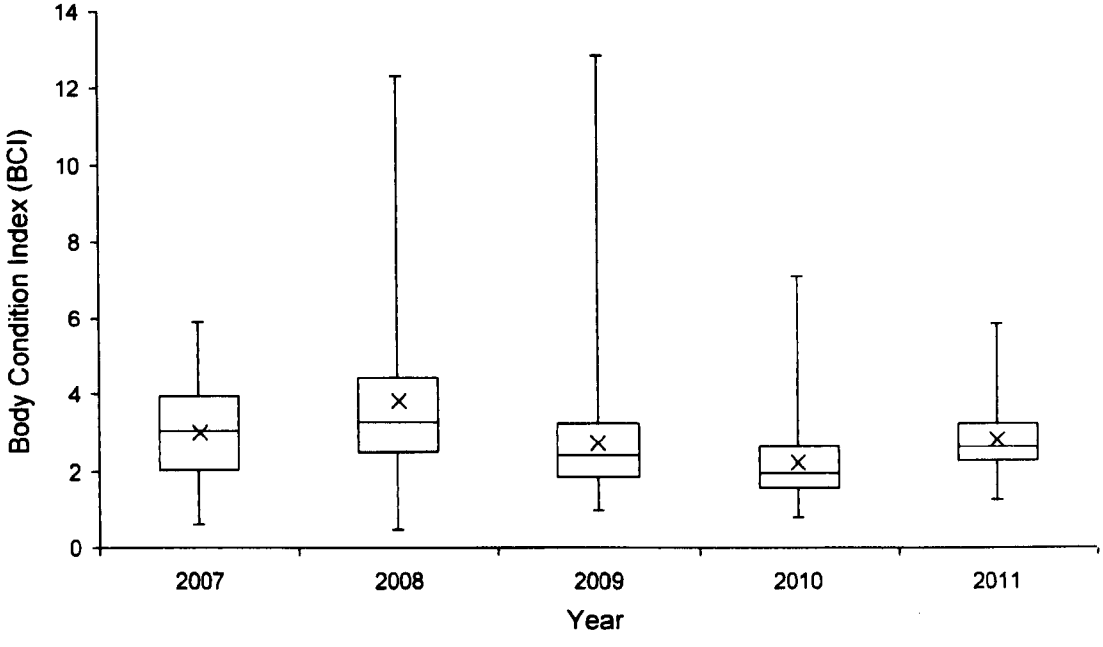


Figure 4.13. Box and whisker plot to show annual body condition of juvenile great crested newts for the period 2008 to 2011. Boxes denote 1st and 3rd quartiles, horizontal lines and crosses denote median and mean values respectively. Whiskers show maximum and minimum BCI.

The mean body condition index of terrestrial juvenile great crested newts varied significantly between seasons (Figure 4.14) ($F_{11,452}$ = 11.7, p < 0.0001). Mean body condition in spring 2008 was highest at 5.9. By summer of that year BCI values had nearly halved to 3.72. The following year BCI scores remained lower than in 2007 with the highest values again in spring at 3.22 and the lowest in summer (2.47). BCI rose slightly in the autumn. By 2010 BCI scores had fallen

slightly from 2008 and showed a similar pattern as in the previous years, though this was less distinct. Values in spring were 2.28 and summer 1.97, whilst autumn levels rose to 2.41. The return of spring saw a slight rise in BCI to 2.80 but no data extend beyond this point to see if scores increased later that year.

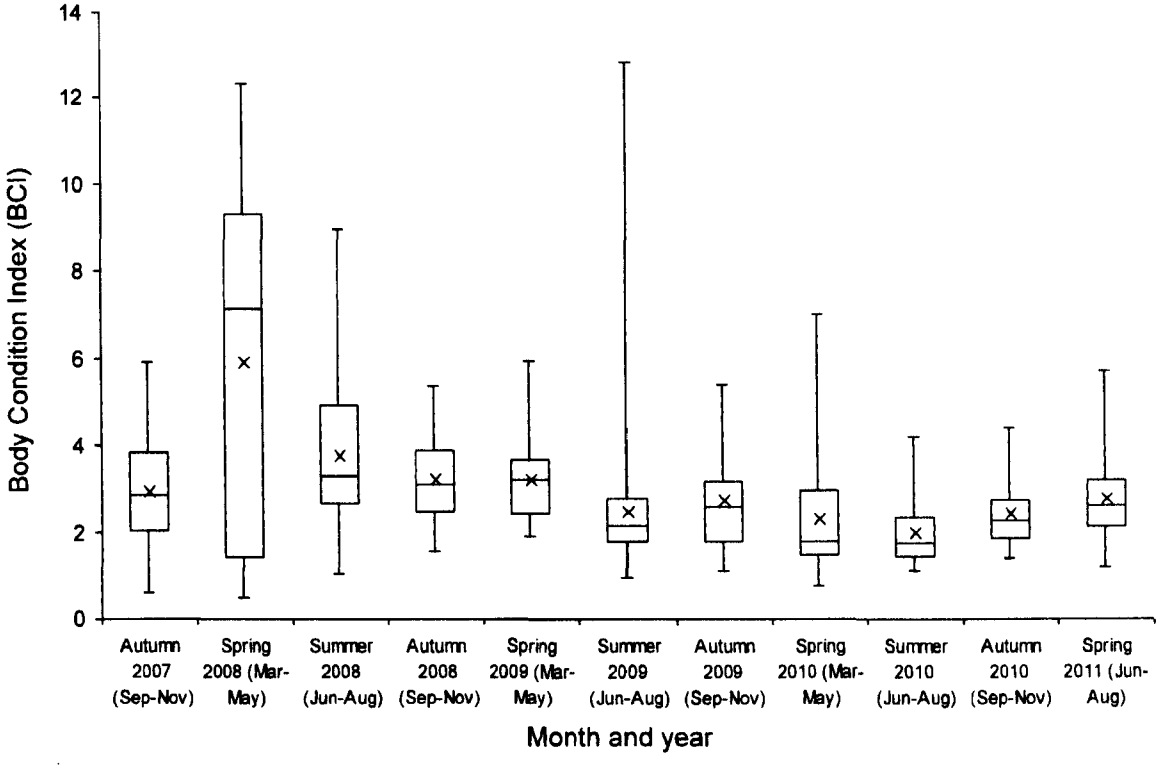


Figure 4.14. Box and whisker plot to show body condition index (BCI) for each season from autumn 2007 to spring 2011. Spring = March to May; summer = June to August; autumn = September to November. Boxes denote 1st and 3rd quartiles, horizontal lines and crosses denote median and mean values respectively. Whiskers show maximum and minimum BCI.

Linear regression showed a slight positive (non-significant) trend between mean monthly winter temperature (December to February) and the body condition of juvneiles the following spring (Figure 4.15) ($R^2 = 0.67$, $F = 4.15$, $p = 0.18$). After the cold winter of 2009 - 2010 juveniles appeared in spring with the lowest mean BCI score of 2.17. In contrast, after the relatively mild winter of 2007 -

2008, juveniles had a mean BCI score of 4.28, nearly double that observed in 2009 - 2010. These results suggest that there may be an influence of winter temperature on juvenile BCI scores in the following spring. However further data are required to determine any further significance in the correlation.

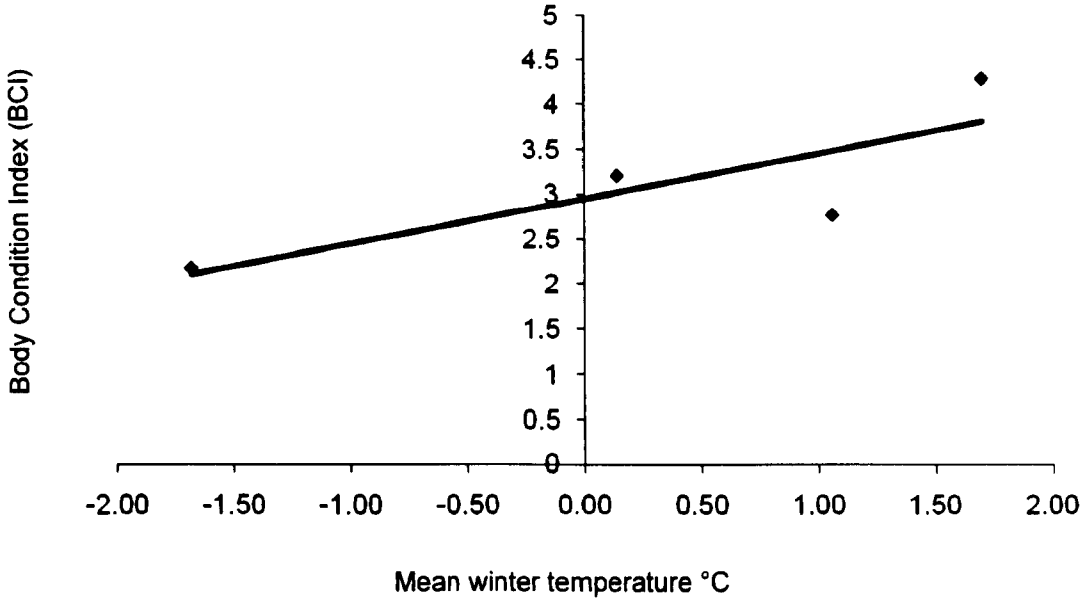


Figure 4.15. Linear regression to show the effect of the previous mean winter minimum air temperature (December to February) and mean body condition of juveniles in spring (March to May).

Linear regression indicated no significant correlation between mean BCI score of juveniles and total non-aquatic rainfall (Figure 4.16) (R^2 = 0.02, F = 0.043, p = 0.86). There was therefore little or no effect of rainfall on body condition of juveniles during the winter months.

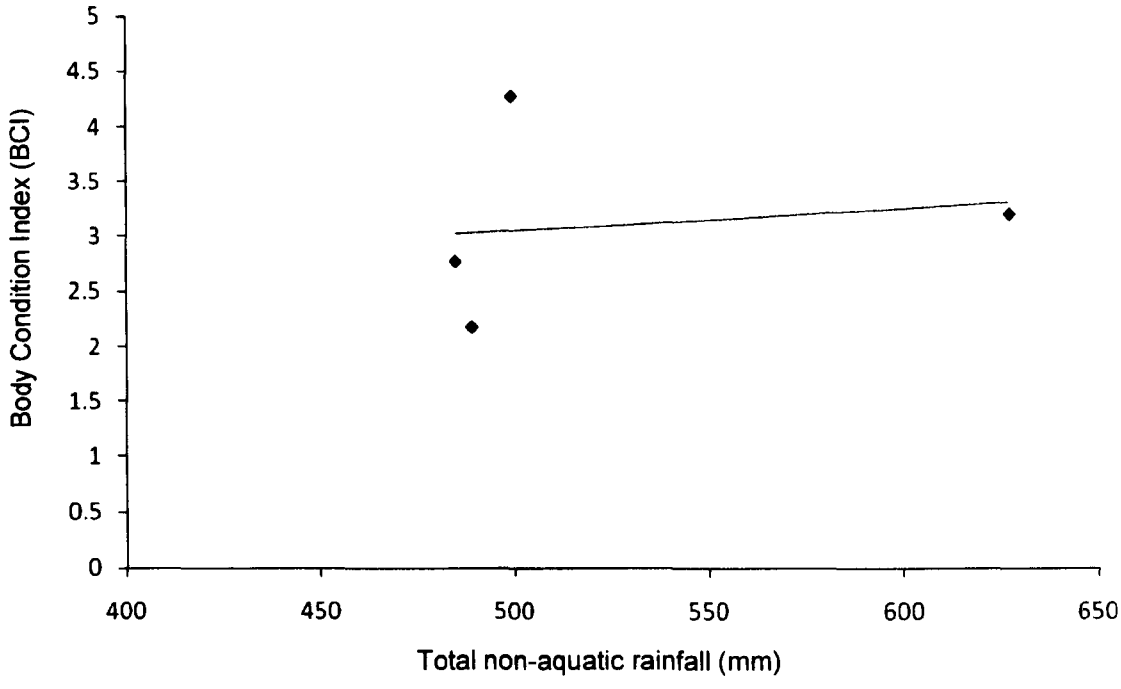


Figure 4.16. Linear regression to show the effect of the previous non-aquatic rainfall (August to February) and mean body condition of juvniles in spring (March to May).

There was a slight non-significant positive relationship between body condition index and growth rates of individuals (Figure 4.17) (R^2 = 0.11, F = 3.89, p = 0.06). This suggests that individuals with a higher body condition may have higher growth rates, but further data are required to determine significance in this relationship.

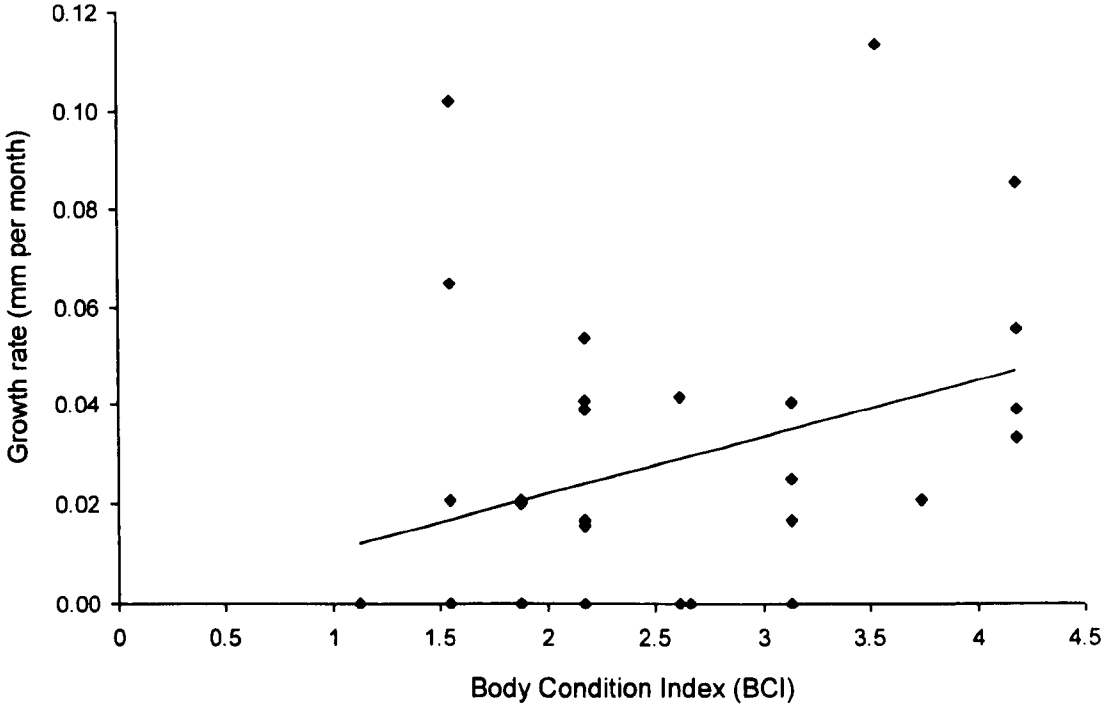


Figure 4.17. Linear regression to show the effect of body condition on growth rate.

4.4.4. Estimation of monthly survival and detection

Model outputs from program MARK for apparent monthly survival and detection show four clear parsimonious models for each year 2008 to 2011. All the highest ranking models had ΔQAICc weights significantly higher than the second ranked model (Table 4.1). The model {Φ(.),p(.)} had the highest ranking result in both 2008 and 2011. Delta QAICc weights for second ranking models were 6.61 and 5.94 respectively indicating the highest ranked model had clear support for the data in every year. This model suggests that apparent monthly survival and detection were constant with respect to time. The model {Φ(.),p(time)} was the most parsimonious in 2009 whereas {Φ(time),p(.)} received the highest rank in 2010. The former indicates that apparent monthly survival remains constant across months but that detection probabilities vary with time, whereas vice versa applies to the 2010 model. Both models received

clear support, though ΔQAICc values were not as high, with second ranking models receiving ΔQAICc values of 2.09 and 3.72 for 2009 and 2010 respectively.

	Model	QAICc	ΔQAICc	QAICc weights	Number of parameters
2008	Φ(.),p(.)	48.97	0.00	0.95697	2
	Φ(time),p(.)	55.58	6.61	0.03517	8
	Φ(.),p(time)	58.58	9.61	0.00785	8
	Φ(time),p(time)	70.74	21.78	0.00002	13
2009	Φ(.),p(time)	54.39	0.00	0.69411	7
	Φ(time),p(.)	56.48	2.09	0.24438	7
	Φ(.),p(.)	59.27	4.88	0.06042	2
	Φ(time),p(time)	67.31	12.91	0.00109	13
2010	Φ(time),p(.)	81.01	0.00	0.85713	8
	Φ(.),p(time)	84.73	3.72	0.13362	8
	Φ(.),p(.)	90.40	9.39	0.00783	2
	Φ(time),p(time)	93.81	12.81	0.00142	13
2011	Φ(.),p(.)	32.43	0.00	0.91846	2
	Φ(.),p(time)	38.36	5.94	0.04714	5
	Φ(time),p(.)	39.01	6.58	0.03414	5
	Φ(time),p(time)	48.82	16.40	0.00025	7

Table 4.1. CJS model selection for apparent *monthly juvenile survival and detection for 2008 to 2011 based upon QAICc in program MARK, Φ = survival, p = detection probability. QAICc = quasi Akaike Information Criteria adjusted using ĉ.*

Apparent monthly survival values in 2008, 2009 and 2011 were constant at 0.56 (C.I. = 0.40 - 0.71), 0.90 (C.I. = 0.63 - 0.98) and 0.77 (C.I. = 0.30 - 0.96) respectively (Table 4.2). This indicates relatively high survival between months in all years, especially 2009 when 90% of juveniles were estimated to have survived the year. Results from 2010 show highly variable estimates for monthly survival ranging from 0.12 (C.I. = 0.03 - 0.37) in October to 1.00 (S.E. = 0) in

April and August. Remaining months had values with large ranges in confidence levels. Estimates for monthly survival in 2009 should therefore be treated with caution due to the high range in survival.

Detection probabilities were constant in 2008, 2010 and 2011 at 0.89 (C.I. = 0.53 - 0.98), 0.89 (C.I. = 0.66 - 0.97) and 0.69 (C.I. = 0.24 - 0.93) respectively. These are relatively high values, indicating high ease in detecting juveniles. Values for monthly detection in 2009 were highly variable ranging from 0.31 (C.I. = 0.06 - 0.74) in September to 1.00 (S.E. = 0) in June. Other months had a range of values from 0.45 to 0.83, all with high confidence levels. As with monthly survival, values for detection probabilities in 2010 should be treated with caution due to the high range in confidence intervals.

4.4.5. Estimation of annual survival and detection

Model outputs from program MARK indicate a clear support for model {Φ(.),p(.)} for the data (Table 4.3). The second ranking model received a ΔQAICc of 3.69, indicating substantial support for the highest ranking model. The model {Φ(.),p(.)} suggests that apparent annual survival and detection probabilities are constant with respect to time.

	Parameter	Estimate	Standard Error	95% Confidence Interval Lower	Upper
2008	1: Φ all months	0.5646832	0.0812194	0.4043415	0.7125476
	2: p all months	0.8876295	0.1000408	0.5252056	0.9825806
2009	1: Φ all months	0.9039172	0.0752497	0.6325881	0.9809175
	2: p March	0.4500833	1836.2740	0.0000000	1.0000000
	3: p April	0.4500833	1836.2740	0.0000000	1.0000000
	4: p May	0.6000000	0.2190890	0.2004055	0.8997718
	5: p June	1.0000000	0.0000000	1.0000000	1.0000000
	6: p July	0.8277595	0.1523851	0.3716724	0.9750280
	7: p August	0.8345245	0.2216396	0.1783221	0.9915394
	8: p September	0.3077438	0.2028752	0.0643327	0.7418900
	9: p October	0.0000000	0.0000000	0.0000000	0.0000000
2010	1: Φ March	0.4500833	0.0000000	0.4500833	0.4500833
	2: Φ April	1.0000000	0.0000000	1.0000000	1.0000000
	3: Φ May	0.7046039	0.2057348	0.2557727	0.9430365
	4: Φ June	0.4973428	0.1810588	0.1930721	0.8035943
	5: Φ July	0.7882230	0.1891418	0.2876991	0.9716696
	6: Φ August	1.0000000	0.0000000	1.0000000	1.0000000
	7: Φ September	0.7630981	0.1508746	0.3855554	0.9429731
	8: Φ October	0.1180150	0.0793262	0.0291674	0.3734075
	9: p all months	0.8952150	0.0702555	0.6631154	0.9737400
2011	1: Φ all months	0.7746525	0.1833570	0.3049293	0.9642043
	2: p all months	0.6910305	0.2112824	0.2433070	0.9396029

Table 4.2. Model output from program MARK showing real function parameters of the most parsimonious model. Standard error and confidence intervals corrected for ĉ: 2008 = 1.17; 2009 = 1.17; 2010 = 1.05; 2011, no adjustment required.

Model	QAICc	ΔQAICc	QAICc weights	Number of parameters
Φ(.),p(.)	77.97	0.00	0.74837	2
Φ(time),p(.)	81.66	3.69	0.11823	4
Φ(.),p(time)	82.09	4.13	0.09513	4
Φ(time),p(time)	83.91	5.95	0.03827	5

Table 4.3. CJS model selection for apparent *annual juvenile survival and detection, 2008 to 2011 based upon QAICc in program MARK, Φ = survival, p = detection probability. QAICc = quasi Akaike Information Criteria adjusted using ĉ.*

Apparent annual survival was estimated at 0.20 (C.I. = 0.12 - 0.30) in all years (Table 4.4), a value lower than that recorded for adults (males: 0.40; females: 0.58) (Chapter 3). Detection probabilities were estimated at 1.0, indicating exceptionally high rates of detection. This indicates extremely high faithfulness to refuges over many months and years by individual juveniles.

			95% Confidence Interval	
Parameter	Estimate	Standard Error	Lower	Upper
1: Φ	0.1976744	0.0462195	0.1221654	0.3037077
2: p	1.0000000	0.0000000	1.0000000	1.0000000

Table 4.4. Model output for from program MARK showing real function parameters of the most parsimonious model. Standard error and confidence intervals corrected for ĉ = 0.16.

4.4.6. Population estimates

Population estimates fluctuated annually ranging from 19 in 2011 to 37 in 2010 (Figure 4.18). The lower value in 2011 may be due to the short field season, which finished in June. The juvenile population was also lower in 2009 when there were estimated to be just 21 individuals.

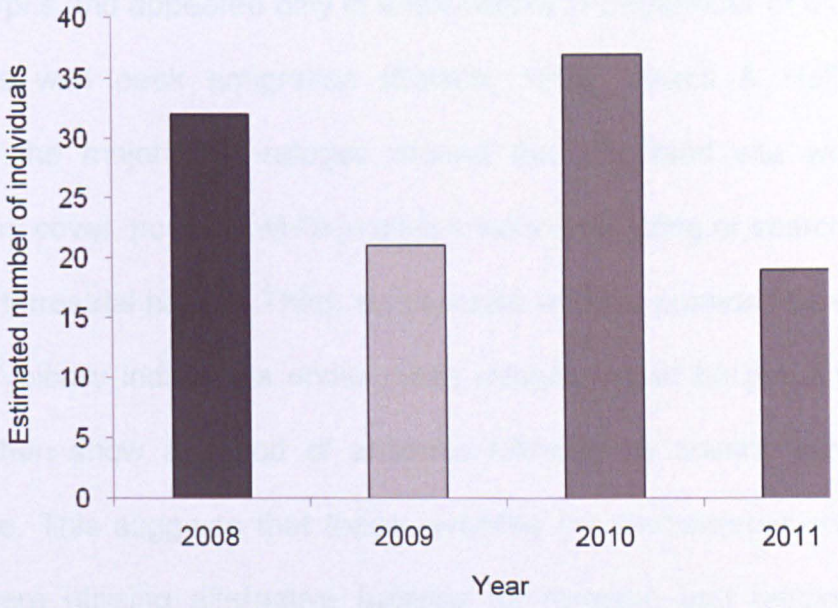


Figure 4.18. Population estimates for juvenile great crested newts 2008 - 2011. There are no standard error values due to detection probability estimates of 1.0.

4.5. Discussion

4.5.1. Patterns and incidence of capture 2008 - 2011

Great crested newts, like many temperate amphibians, require damp conditions to avoid desiccation (Marty et al., 2005) and due to a large surface-area to volume ratio juveniles may be more susceptible to drying than adults. The concrete refuges in this study may have been preferred by great crested newts as they were heavier than logs with a closer fit to the substrate underneath resulting in damp leaves and soil all year round. Although larger refuges may also retain more moisture, these were no more favourable than smaller ones.

This indicates that substrate type, rather than size, may have more impact in microhabitat selection by juveniles.

Juveniles in this study appeared to utilise terrestrial cover objects in three different ways. First, refuges were used as a temporary resting place whilst juveniles were emigrating from ponds. These individuals were usually metamorphs and appeared only in a few weeks in September or October, which coincides with peak emigration (Sinsch, 1997; Verrell & Halliday, 1985). Second, the majority of refuges around the woodland site were used as temporary cover, possibly while juveniles were emigrating or searching for more suitable terrestrial habitat. Third, six concrete refuges provided semi-permanent cover. Typically individuals under these refuges would be present for several weeks then show a period of absence followed by several more weeks of presence. This suggests that these juveniles (no metamorphs were recorded here) were utilising alternative habitats for foraging and feeding and using concrete slabs for refuge. Jahn (1995) and Griffiths (1984) observed similar behaviour in adult great crested and smooth newts respectively. Individuals regularly moved short distances between refuges, usually returning to one specific location. Verrell & Halliday (1985) noticed juvenile great crested newts emigrating to and from a breeding pond where they would feed and gain weight. This behaviour was only observed at one pond over a single breeding season and may have been unique to this site. The terrestrial conditions at my study site were very good with lots of foraging habitat so it seems likely that when juveniles were absent from refuges they were utilising alternative terrestrial habitats within the woodland area.

Only 0.7% of juveniles were found in consecutive years indicating that juveniles had either temporarily or permanently emigrated from the study site (Bailey et al., 2004b), were utilising alternative refuges, had returned to ponds (Baker, 1999), or had died. Juveniles disappeared from refuges from the end of November until early February. Griffiths (1984) suggested that this was due to vertical migration of newts into the soil. In common with terrestrial-living adult smooth newts (Griffiths, 1984), numbers of juveniles in my study increased in early spring to a peak in late spring or early summer. This was probably due to the increase in temperature resulting in more activity and feeding (Arntzen, 2000). In two years (2008 & 2010) there was a second peak in abundance in August/September. This corresponded with metamorph emergence from ponds and was linked to higher captures of individuals under logs surrounding ponds.

Juveniles were significantly more likely to be encountered under cover objects in warmer weather conditions. Griffiths (1984) found a similar trend in smooth newts and several other studies on amphibians have shown a similar relationship between amphibian movement and temperature with individuals starting to move when temperatures reach 4 - 5 °C (Andreone & Giacoma, 1989; Latham & Oldham, 1996; Verrell & Halliday, 1985). In ectotherms, metabolic rate is affected by ambient air temperature (Holenweg & Reyer, 2000), resulting in lower activity in colder conditions (Grist, 1994; Reading, 1998). There was no significant relationship between monthly rainfall and juvenile movement. This is similar to observations in other northern European *Triturus* newt species (Griffiths, 1984; Reading, 2007; Verrell & Halliday, 1985), but contrary to many North American and southern European amphibians (e.g. Andreone & Giacoma, 1989; Forester et al., 2006; Kleeberger & Werner, 1982;

Mazerolle, 2001). The lower rainfall characteristic of these countries may make moisture levels more critical for movement.

4.5.2. Growth rate

Juvenile growth rates were fastest only in the smallest individuals (35 to 39 mm), which were usually metamorphs. Above 44 mm, growth rate significantly declined. A few studies have demonstrated a decrease in growth rate with increasing age (Arntzen, 2000; Sever et al., 2001). Arntzen (2000) found that great crested newts had fastest growth in the first three years of life and only in the fourth year did growth rate decline where as Sever et al. (2001) and Homan et al. (2003) recorded fastest growth in the first year of life in the smooth newt and spotted salamander *Ambystoma maculatum* respectively. This discrepancy in findings may be due to different biotic and abiotic factors operating in different populations (Hemelaar, 1988; Miaud et al., 2001) resulting in changes in availability and quality of food, habitat quality, foraging behaviour and climate (Arntzen, 2000).

Results from my study show that growth rates were positively associated with rainfall. This may be due to an increase in activity after rainfall, resulting in increased foraging and growth (Cooke, 1986). Neither time of year nor air temperature affected growth rate. This is contrary to that of Arntzen (2000) who found growth in great crested newts was highest during spring and summer and that of Jakob et al. (2002) who noted slowest growth in the autumn and winter.

4.5.3. Body condition

Body condition in juveniles was highest in spring and lowest in summer before rising again in autumn in all years. Arntzen et al. (1999) also noted higher BCI values in spring compared to other months in an adult great crested newt population. Similarly Baker (1992) reported that male great crested newts had a higher body condition after the winter. Lower BCI values in the summer may reflect drying of the soil and surrounding microhabitat, resulting in fewer feeding opportunities or decreased availability in terrestrial invertebrates. Amphibians generally prefer damp and warm conditions (Cooke, 1986; Reading, 1998) and are not suited to long periods without rain. In Mediterranean countries salamanders often aestivate in summer months (Olgun et al., 2005), which serves to reduce moisture loss. Therefore during June to August, juveniles may remain under refuges and rely on fat reserves, resulting in a lowering of body condition. Rising body condition in the autumn months may reflect the return of cooler, damper conditions and greater opportunities to forage and feed in preparation for winter.

Body condition varied considerably between years and results from this study showed that temperature and rainfall did not have a direct influence on body condition. Body condition was highest after the mild winter of 2007/08 and lower after the colder winters of 2009/10 and 2010/11. This is contrary to research by Băncillă et al. (2010), Griffiths et al. (2010), Holenweg & Reyer (2000), and Reading (2007) who all found that body condition in adult amphibians was lower after milder winters possibly because individuals use more fat resources in mild temperatures due to an increased metabolic rate. Juveniles are smaller than adults and may not be able to store enough resources to allow survival through

the winter. Therefore juveniles may rely on greater levels of feeding during the winter months than is required by adults. Milder temperatures have been observed to increase foraging activity in common toads (Grist, 1994). An increase in foraging in milder winters may lead to greater opportunities for great crested juveniles to feed and result in a higher body condition. In colder winters, less activity would lead to reduced food intake and a subsequent fall in BCI values. The lower body condition observed in 2009 and 2010 may therefore reflect lower foraging opportunities after the longer, colder winters. Reading (2010) observed higher body condition in common toad metamorphs emerging from ponds after warm summers, which was due to increased feeding as tadpoles. Terrestrial juveniles may therefore be affected by environmental conditions, which have an impact on feeding and BCI values.

4.5.4. Estimation of monthly and annual survival, detection probabilities and population size

Monthly survival of juveniles was highest in the period March to October which is expected in a species which is distasteful to predators (Verrell, 1985) and protected under concrete refuges. Lower survival later in the year may reflect higher mortality in the vulnerable late-emerging metamorphs. The relatively high values for monthly detection may reflect specific microhabitat conditions under the 6 concrete slabs, which encourages high site fidelity in juveniles.

Annual survival of great crested juveniles was constant in all years at 0.19. This estimate is lower than the value of 0.59 previously recorded in juvenile great crested newts by Baker (1999) which was based on counts from aquatic environments rather than in terrestrial habitats. Adult survival is generally higher

than that of juveniles (Arntzen & Teunis, 1993; Baker, 1999; Griffiths et al., 2010), probably because adults are less vulnerable to factors including predation, lack of food and weather conditions. Estimates of apparent survival cannot distinguish between permanent emigration and death, resulting in potentially incorrect estimates of survival (Schmidt et al., 2007). Therefore any permanent emigration by juveniles to alternative sites would have resulted in a low estimate for survival. Juveniles may also have undergone temporary emigration for a few years before returning as adults which has been observed in other amphibian populations (Bailey et al., 2004a, 2004b; Kendall et al., 1997; Malmgren, 2001; Schaub et al., 2004; Schmidt et al., 2002) and which may have been a potential cause of bias in survival estimates in this study.

Many studies indicate that juvenile survival is highly variable (Arntzen & Teunis, 1993; Kupfer & Kneitz, 2000) but my results indicate that apparent survival in juveniles is constant across years. These findings, along with the constant adult apparent survival calculated in the adult population (Chapter 3), suggest that variations in adult and juvenile population size may either reflect variations in larval recruitment within ponds (Griffiths, 1997) or different levels of emigration in particular juvenile cohorts.

4.5.5. Implications of findings

- The use of concrete cover objects may be useful in providing suitable terrestrial microhabitats for juveniles to utilise in mitigation projects, especially after newts are moved to newly created sites.
- Management practices should provide opportunities for juveniles to forage and feed, particularly in spring months to allow BCI levels to rise in preparation for the summer. This would involve ensuring suitable terrestrial habitats and cover are in place before juveniles emerge from hibernation at the end of winter.
- Provision of suitable foraging and refuge habitats is important to enhance long-term population persistence through continued higher survival in juveniles.
- Adult (Chapter 3) and juvenile survival was found to be constant between years. Therefore a focus on enhancing larval recruitment may need to be a particular focus for management practices in this species.

4.5.6. Critique of methods

1. This study was conducted over a relatively small area in one location. Results may therefore only be applicable to this particular habitat and site so caution may need to taken if applying findings here with other localities.

2. Juveniles were not studied over the winter months (December to February) due to lack of captures, probably as a result of vertical migration. This resulted in a lack of data in juvenile growth rates and body condition over the winter period.

3. The smallest metamorphs found surrounding ponds could not be identified as individuals due to a lack of belly pattern (as a result of small size and young age). Therefore it is possible that some of these individuals were recaptured but misidentified in future years. This would have had impact on juvenile survival estimates.

4. Juveniles of several species are known to move widely after emerging from ponds (Hayward, 2010), leading to high levels of temporary and permanent emigration. Since these parameters remained under estimated, this would have led to lower estimates of juvenile apparent survival and population size.

4.6. Conclusions

1. Juvenile great crested newts selected a variety of terrestrial cover objects with concrete slabs being utilised significantly more frequently than natural logs and stones.

2. Individuals utilised terrestrial cover objects as a: i) temporary refuge for juveniles emigrating from ponds; ii) refuge in deciduous woodland for a short-term basis, possibly for emigrating juveniles; iii) semi-permanent refuge for some individuals for many months.

3. Number of captures was positively correlated with mean monthly air temperature but not rainfall, which indicates that the best times for detecting juveniles are when air temperatures rise in spring months.

4. Some juveniles appeared to emigrate away from ponds to new habitats while others stayed close to ponds and utilise small home ranges for feeding. These individuals often used the same refuge for many months in one year.

5. Juvenile growth rates in this study were fastest when in the smallest (35 - 39 mm) size category, after which growth rates significantly declined. Growth rates were faster after periods of rainfall.

6. Juvenile body condition was highest in spring and lowest in summer indicating that foraging and feeding during the early part of the year is important in building up body condition for the summer months.

7. There was a negative (non-significant) relationship between mean winter minimum air temperature and body condition of juveniles the following spring. This suggests that juveniles emerge from winter hibernation in a higher body condition after warmer winters but more data is required to determine whether this becomes a significant trend. There was no effect of winter rainfall on juvenile body condition.

8. There was no significant relationship between body condition and growth rates, though there was a slight positive trend. This suggests that individuals with a higher body condition may have faster growth but further data are required to fully understand this relationship.

6. Apparent monthly survival of juveniles on land was relatively high, indicating that between spring and autumn juveniles had a high chance of survival.

Results suggest that once in their terrestrial phase rates of mortality decline significantly compared to that of larvae.

7. Apparent annual survival of juveniles was constant between years and relatively low (0.19) which may be due to higher levels of permanent emigration and predation compared to adults.

8. Population sizes of juveniles varied between years probably due to a combination of variation in larval recruitment or annual changes in the degree of emigration, rather than changes in levels of juvenile survival.

5. Factors affecting the body condition in a great crested newt *Triturus cristatus* metapopulation.

5.1. Abstract

Estimation of body condition index (BCI) may give valuable information on the fitness and reproductive potential of individuals. Body condition index scores were calculated for 995 individual great crested newts *Triturus cristatus* captured from four ponds over a five-year period. Male, but not female, body condition varied significantly between ponds. BCI scores in both sexes varied significantly by year with opposite trends. Males exhibited lowest body condition in March followed by a rise in the period March to May then a fall in June in three out of five years. Females had high BCI in spring which fell to lower levels later in the season within each year. Male BCI scores exhibited a slight negative (non-significant) relationship with mean winter minimum air temperature. Female BCI scores showed the opposite, non-significant trend. There was a slight negative (non-significant) relationship between BCI and rainfall in males suggesting low BCI after wetter winters. There was no effect of non-aquatic rainfall on BCI scores in females. Findings indicate that BCI varies considerably due to a range of factors and that long-term studies are required to determine whether changes in winter climatic conditions significantly affect great crested newt body condition.

5.2. Introduction

The use of Body Condition Index (BCI) scores to assess the physiological state of an individual has been widely applied in many studies of animal populations including amphibians (Cooke & Arnold, 2003; Kopecký et al., 2010), seabirds (Le Bohec et al., 2007; LormÉe et al., 2003), and mammals (Schulte-Hostedde et al., 2001). Body Condition Index scores, which are based on mass/length regressions, may give an indication of the fat reserves of an animal and reflect its underlying health and susceptibility to disease (Janin et al., 2011). BCI scores may also indicate past foraging success, fighting ability and the capacity to cope with environmental pressures, which may impact on reproductive success (Jakob & Marshall, 1996). However, measurements of body condition in vertebrates assume that length/mass gives a direct indication of levels of body fat (Green, 2001) and this may not be true in all individuals. For example in female amphibians, BCI scores may simply reflect the capacity to hold eggs since larger females are more fecund (Cooke & Arnold, 2003; Halliday & Tejedo, 1995; Malmgren & Thollesson, 1999). Therefore values for BCI in female amphibians are more likely to indicate their level of fecundity. Also, amphibians hydrate when entering the water which will affect mass and thus BCI scores (Griffiths, 1996). In addition, microhabitat conditions during the non-breeding season may influence gonad development and fat storage (Semlitsch et al., 1993), which may result in variation in BCI values at different times of the year. Therefore caution must be taken when drawing inferences from BCI calculations since values will not always reflect actual body condition.

In a study of great crested newts, Arntzen et al. (1999) found that body condition scores for great crested newts fluctuated significantly during the

breeding season with the highest values in spring. The first individuals at ponds had higher body condition than later arrivals. This implies a high energy to mass expenditure during the aquatic reproductive phase. A study by Sztatecsay & Schabetsberger (2005) on common toads *Bufo bufo* revealed that body condition scores varied considerably between sites, resulting in differential survival in terrestrial habitats. These studies show that BCI may change with seasons, sites and biotic factors such as competition.

The long-term effects of non-breeding weather conditions on subsequent body condition and survival during the breeding season have been documented in many animal populations including birds (Ahola et al., 2009; Najmanová et al., 2009; Wright et al., 2009) and mammals (Burek et al., 2008; Rödel et al., 2004; Weladji & Holand, 2003). Studies on amphibian populations suggest that mean winter temperatures have an effect on survival (Anholt et al., 2003; Araújo et al., 2006) with varying effects, depending on location and species ecology. Several studies demonstrate a negative relationship between mean winter temperature and body condition the following spring. For example, Băncillă et al. (2010) found that yellow-bellied toads *Bombina variegata* emerged from winter hibernation in higher body condition after colder winters. In the British Isles, Reading (2007) and Griffiths et al. (2010) recorded lower body condition in common toads and great crested newts respectively after milder winters. This may be because individuals expend more body fat reserves in higher temperatures due to an increased metabolism (Reading, 2007). These studies have all been carried out over periods of more than 10 years. Few studies have determined whether the effects of winter temperature on body condition can be detected over a shorter time period.

Additional information on the body condition of great crested newts will be highly informative in highlighting possible effects on population dynamics. In this study I examined body condition index scores of adult great crested newts from four breeding ponds over a five-year period. Specifically, I wanted to address the following questions:

i) Does BCI score vary between sites? The four study ponds are all within 500m of each other and I wanted to determine whether subtle differences within each breeding site had an effect on BCI scores.

ii) Does BCI score vary with season and year? Over five years, differences in biotic and abiotic conditions may vary within ponds resulting in a difference in BCI scores.

iii) Is there a relationship between winter temperatures and rainfall and BCI scores of adults in spring? I wanted to determine whether the trends observed by Reading (2007) and Griffiths et al. (2010) were observable in a different metapopulation over a shorter time period.

5.3. Methods

5.3.1. Capturing great crested newts

Adult great crested newts were captured in funnel traps as described in the general methods (Chapter 2). Data from five field seasons were grouped by year, month and pond for analysis.

5.3.2. Data analysis

There are three commonly used methods for calculating BCI scores: 1) Ratio index, which is the simplest and involves taking body mass and dividing by the cube of the body length (Wheeler et al., 2003); 2) Slope adjusted ratio index, which uses a 'standard' population to generate the slope of the regression. Captured individuals are then compared to this line; 3) Residual index or ordinary least squares method (Reading, 2010), where body mass is regressed on body size after data are transformed to meet assumptions of the regression. The residual distances of individual points from the regression line can then serve as estimators of condition (Jakob & Marshall, 1996). The most widely used method in amphibian studies is the latter, using snout-vent length (SVL) and mass, although there are several assumptions which are: 1) Mass increases linearly with SVL; 2) SVL is an accurate measure of actual structural size; 3) True condition is independent of SVL (Green, 2001). Since these assumptions may not always be met, an alternative measurement is the Density Body Condition (DBC) calculation which uses a measure of the volume of the body, rather than simply body length since this is where most resources are stored (Moya-Larani et al., 2008). However this is more difficult than SVL to measure accurately so given the practical constraints of time and resources, the

residual index method using SVL and mass as variables were used in this study.

Male and female great crested newts were grouped separately for BCI calculation due to differences in size (Verrell & Halliday, 1985). Adult body condition index was calculated using the residuals (y) from a type I ordinary least squares regression of mass against SVL after log transformation using the formula:

$$BCI = (Log_{10} SVL) / (Log_{10} mass)^{y}$$

Body mass indices were calculated for four months for males, March to June for each year 2007 to 2011. Each capture and recapture was treated separately since BCI values for each individual changed across seasons and years. Females lay eggs through the period March to June (Langton et al., 2001) so to avoid a large drop in BCI score due to egg loss, BCI was only recorded for the period March to end of April for each year 2007 to 2011. Although BCI loss due to egg loss may occur during this period, this would be minimised.

An unbalanced two-way analysis of variance (ANOVA) was performed using the general linear model (GLM) facility in Minitab to determine whether there was a difference in BCI with year, site and season. Due to the low sample size from Speakman's pond (n = 16) data was not included in the (ANOVA) analysis.

5.3.3. Weather data

Weather data were collected from an on-site meteorological station as stated in the general methods (Chapter 2). Mean winter (November to February) minimum air temperature and non-aquatic rainfall (November to February) were

calculated since adults are typically in their terrestrial phase during this period (English Nature, 2001a; Baker et al., 2011).

5.4. Results

5.4.1. Differences in body condition between ponds

A total of 995 adults (575 male, 420 female) were captured in the period 2007 to 2011. Male body condition did not vary significantly between ponds (Figure 5.1) ($F_{2,540}$ = 0.74, p = 0.48). Frog Pond had the lowest mean body condition index of 2.86 (S.E. = 0.08) compared to Lily Pond, which had the highest value of 3.34 (S.E. = 0.16). Oak plain had mean BCI scores between those of the other two ponds (3.16; S.E. = 0.09).

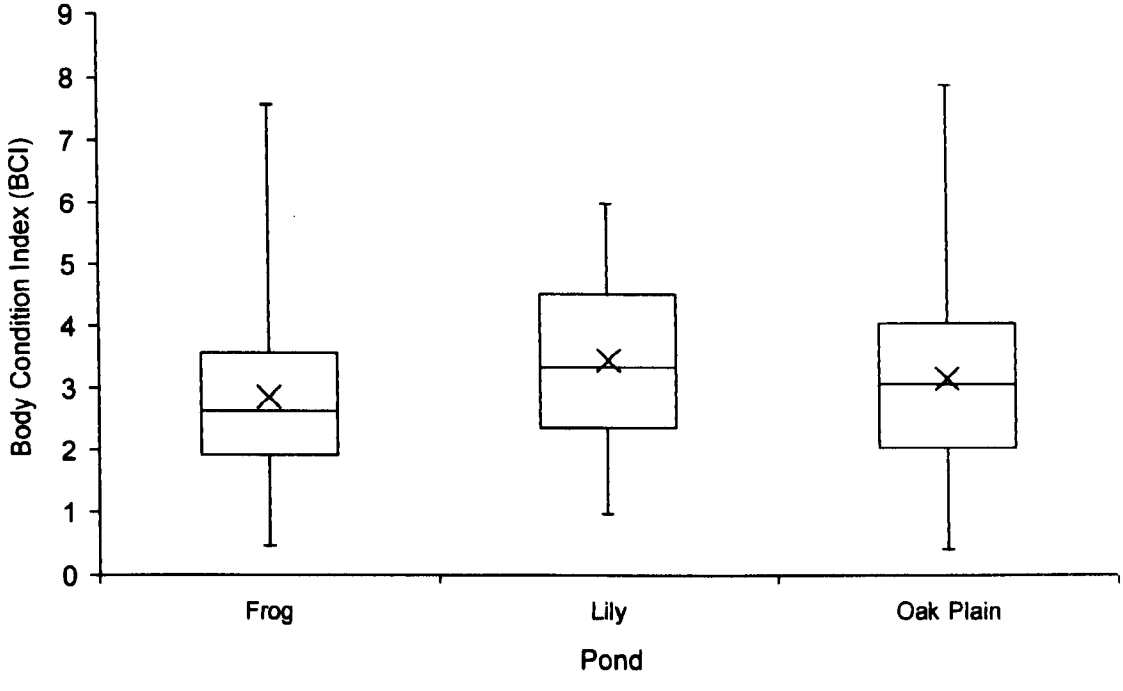


Figure 5.1. Box and whisker plot to show Body Condition Index (BCI) of adult males in each study pond over the period 2007 - 2011. Boxes denote 1st and 3rd quartiles, whiskers maximum and minimum BCI values and crosses mean values.

There was no significant variation in female body condition between ponds (Figure 5.2) ($F_{2,402}$ = 2.53, p = 0.08). In Frog, Oak Plain and Speakman's ponds mean BCI scores were very similar at 2.86, 3.01 and 3.11 respectively. Females in Lily Pond had a slightly lower BCI than the others at 2.20, though this was based on a smaller sample size (n = 13). Females had the highest mean BCI in Speakman's Pond, though again this was based on a small sample size (n = 16). Results for Lily and Speakman's ponds are therefore likely to be less representative of the pond population.

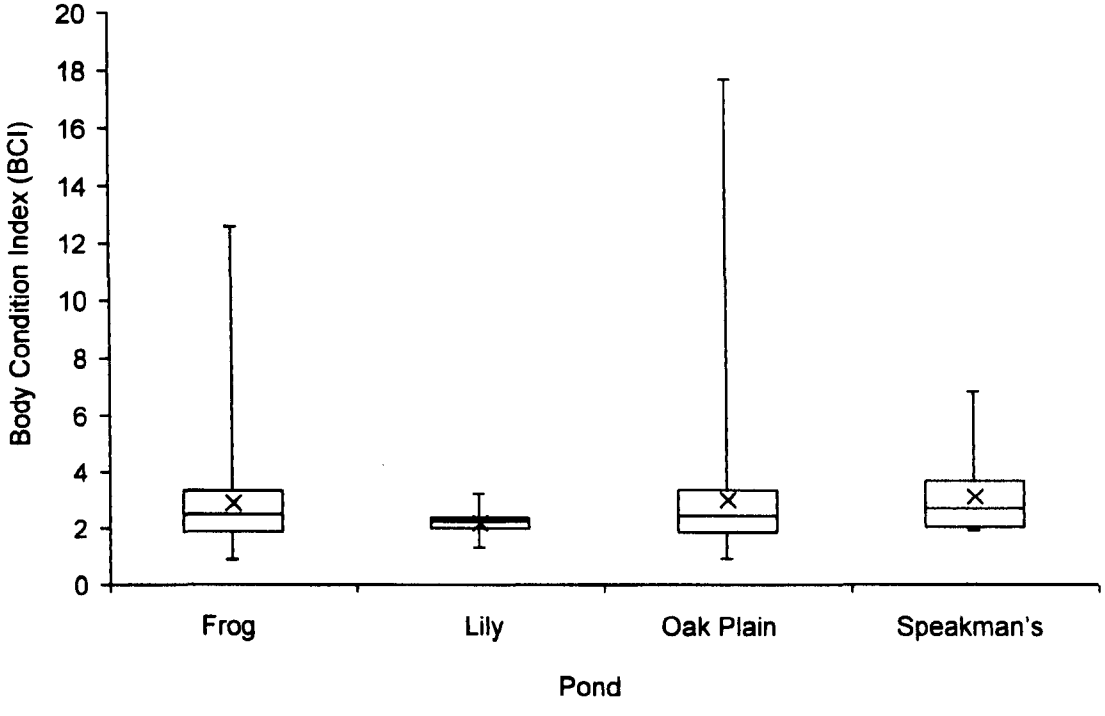


Figure 5.2. Box and whisker plot to show Body Condition Index (BCI) of adult females in each study pond over the period 2007 - 2011. Boxes denote 1st and 3rd quartiles, whiskers maximum and minimum BCI values and crosses mean values.

5.4.2. Differences in body condition between years

Adult male great crested newts showed significant variation in body condition index scores between years (Figure 5.3) ($F_{4,540} = 6.61$, $p < 0.0001$). Post hoc ANOVA revealed a significant difference in BCI scores between the years 2007 to 2009 ($F_{2,488} = 73.78$, $p < 0.0001$) but not between 2009 and 2011 ($F_{2,181} = 2.00$, $p < 0.19$). Mean BCI scores were significantly lower in 2007 at 2.06, compared to a range of 2.78 to 3.74 in the period 2008 to 2009. BCI scores were highest in 2008 at 3.74. This coincided with relatively mild winter and average rainfall (Figure 5.4). BCI values dropped slightly in the period 2009 to 2010 during a period of relatively cold winters.

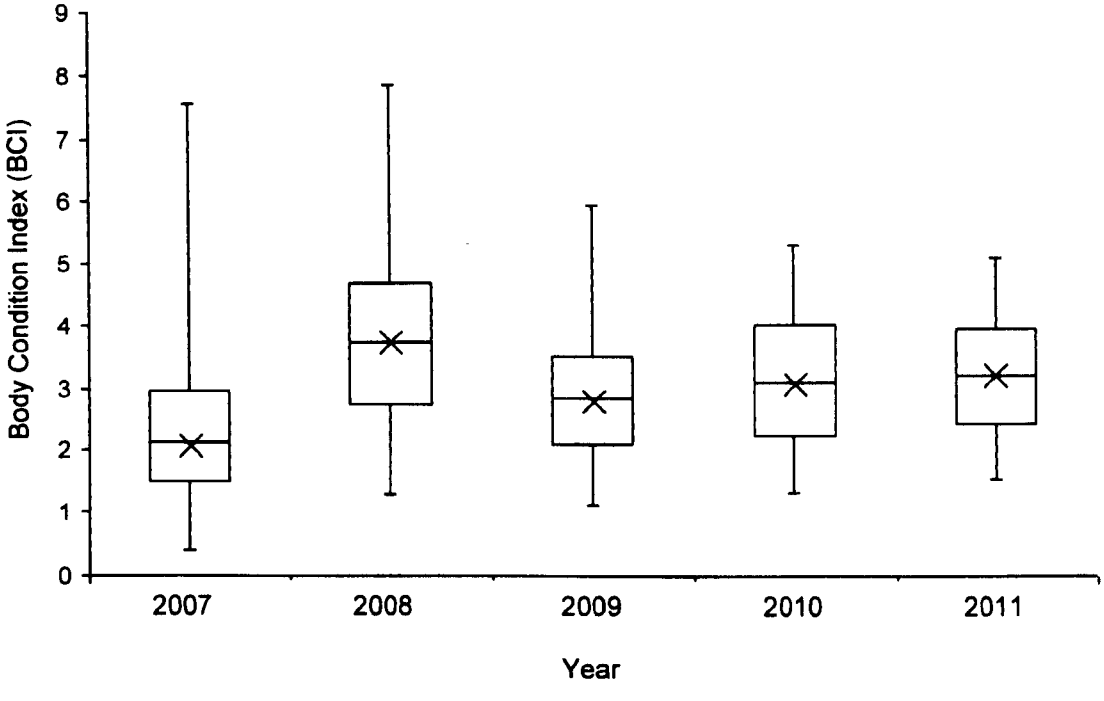


Figure 5.3. Box and whisker plot to show Body Condition Index (BCI) of adult males in each study year, 2007 - 2011. Boxes denote 1st and 3rd quartiles, whiskers maximum and minimum BCI values and crosses mean values.

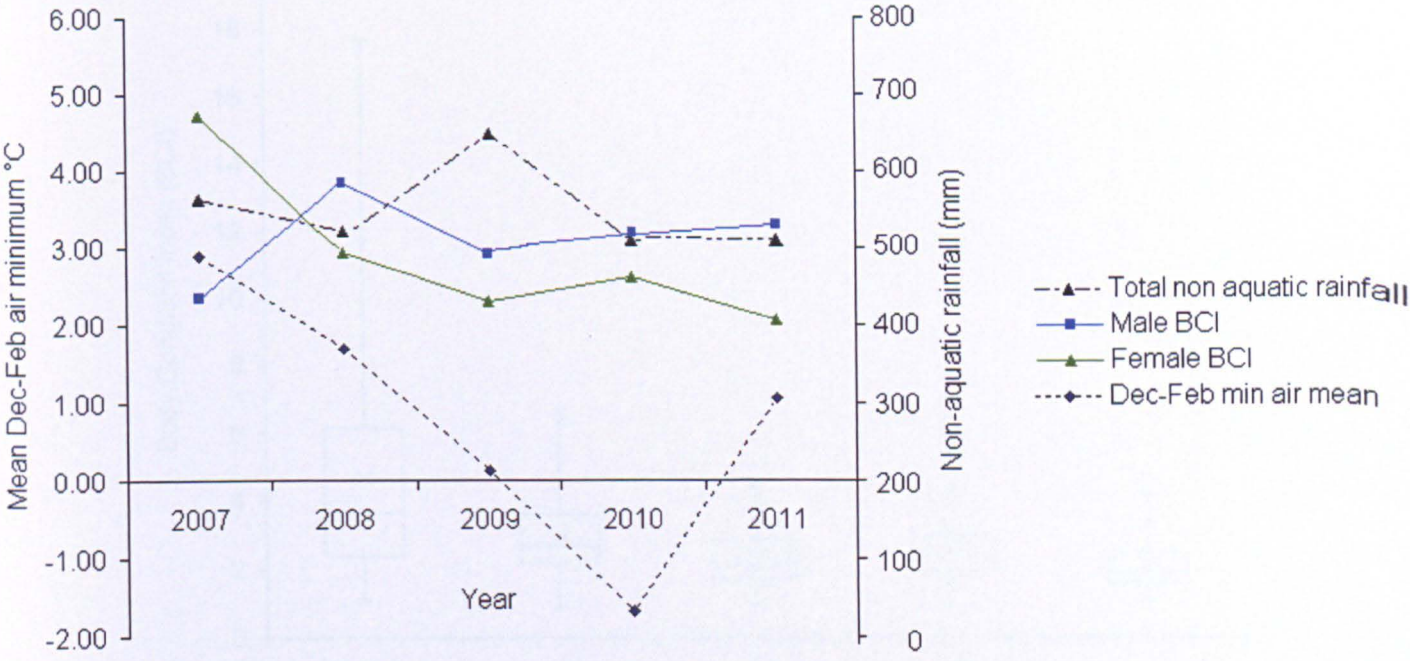


Figure 5.4. Male and female BCI plotted with total non-aquatic rainfall and December to February minimum air mean temperature.

Body condition index scores were significantly different across years in females (Figure 5.5) ($F_{4,402}$ = 19.52, $p < 0.0001$). Post hoc ANOVA revealed a highly significant difference in BCI scores between 2007 and 2008 ($F_{1,171}$ = 27.68, $p < 0.0001$) as well as in the period 2008 to 2011 ($F_{3,355}$ = 10.31, $p < 0.0001$). The pattern of BCI scores was different to that exhibited by males. The highest mean BCI score was in 2007 at 4.71, the opposite for that shown by males. Females in the period 2008 to 2011 had more consistent mean BCI scores ranging from 2.31 to 2.94. The lowest BCI score was in 2011 at just 2.31, after a cold and relatively dry winter (Figure 5.4). Body condition appeared to decline through the study period 2007 to 2011. It was not possible to determine whether this was a temporary trend.

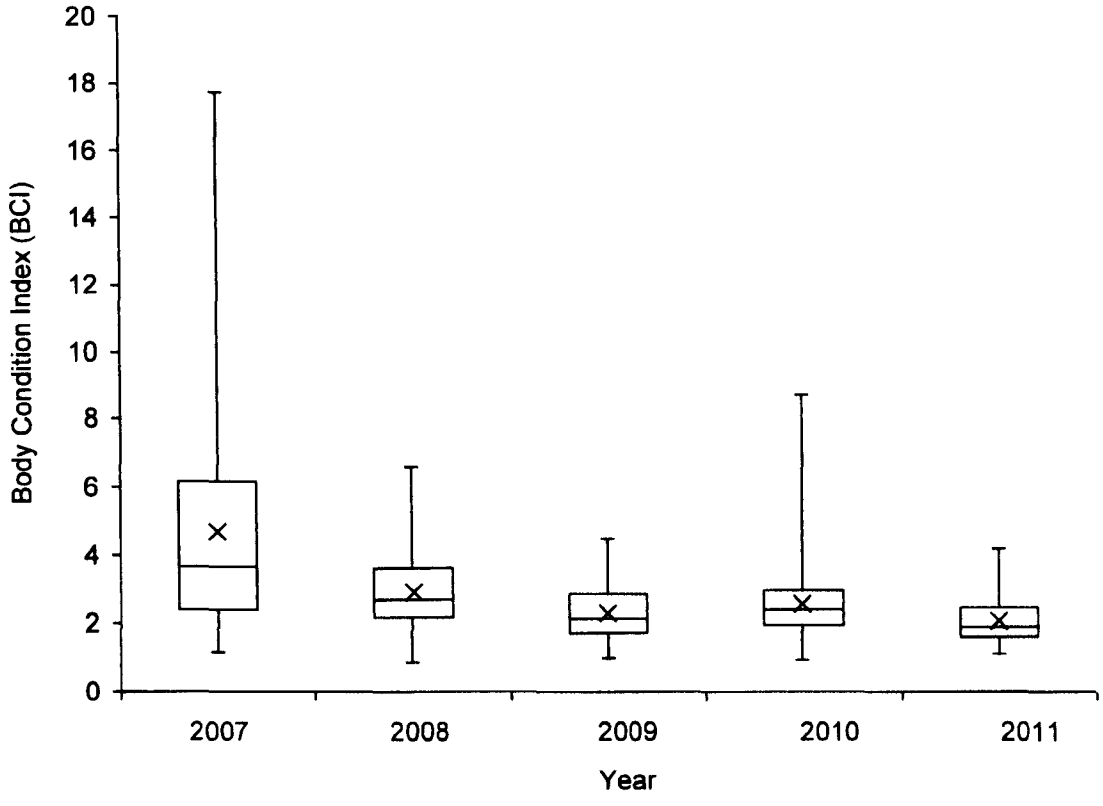


Figure 5.5. Box and whisker plot to show Body Condition Index (BCI) of adult females in each study year. Boxes denote 1st and 3rd quartiles, whiskers maximum and minimum BCI values and crosses mean values.

5.4.3. Differences in body condition between seasons

Within each year, male body condition varied significantly between months (Figure 5.6) ($F_{3,540}$ = 5.36, p = 0.01). Mean BCI scores increased through the months March to May 2007 to 2009. This pattern was not as evident in the remaining years 2010 & 2011. BCI scores also fell in June 2008, 2009 & 2011 but not the remaining years. These results show that BCI is highly variable between months but overall appears to show an increasing trend through the breeding season, often with a fall in June.

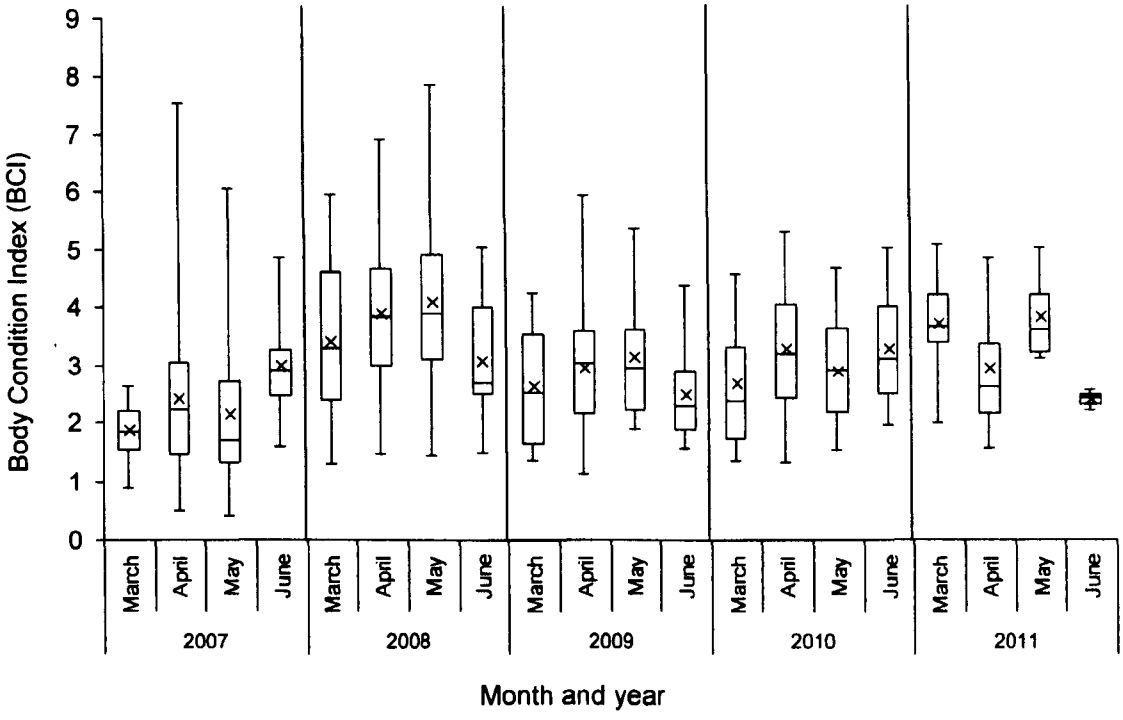


Figure 5.6. Box and whisker plot to show Body Condition Index (BCI) of adult males in each month of the breeding season, March to June. Boxes denote 1st and 3rd quartiles, whiskers maximum and minimum BCI values and crosses mean values.

There was no significant difference in female BCI across the season (Figure 5.7) ($F_{1,402}$ = 1.86, p = 0.17). Across each season BCI appeared to fall in all years except 2009. The largest drop occurred in 2007 with mean BCI values falling from 6.26 to 3.98 from the beginning of March to the end of April. Decreases across the season in 2008, 2010 and 2011 were less marked. In 2009 BCI scores increased from March (2.18) to early April (2.68) before falling again by the end of the month (2.18).

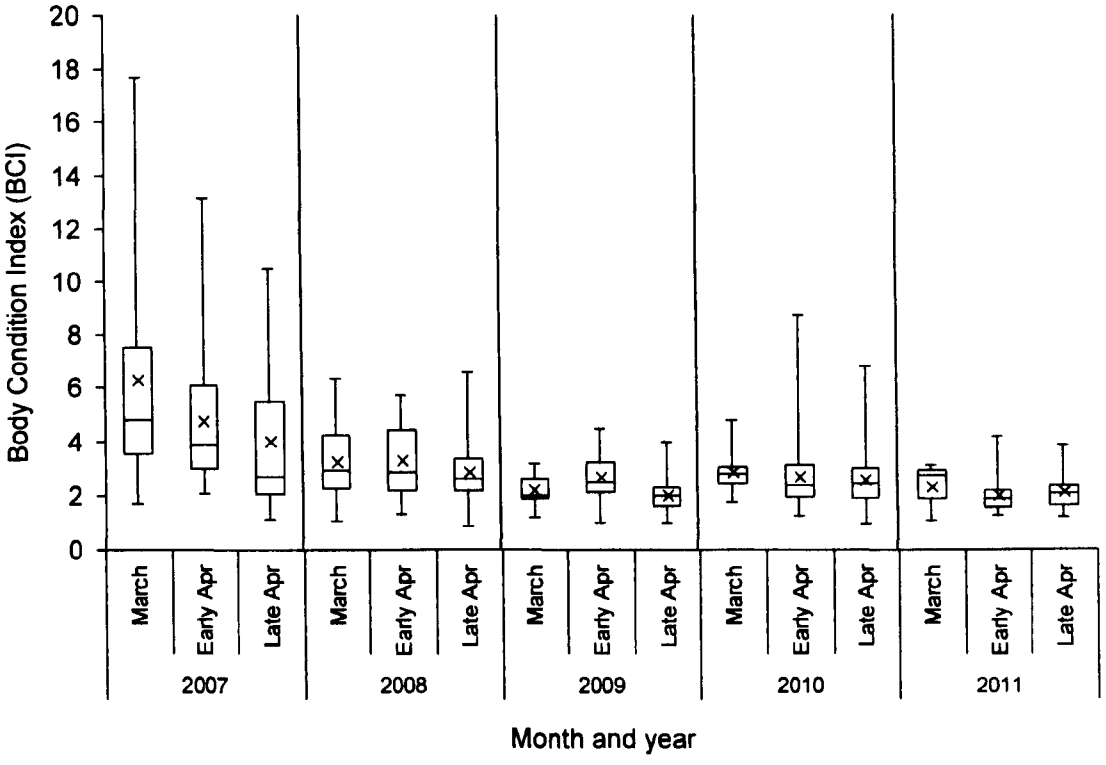


Figure 5.7. Box and whisker plot to show Body Condition Index (BCI) of adult females in the early part of each breeding season, March to late April. Boxes denote 1st and 3rd quartiles, whiskers maximum and minimum BCI values and crosses mean values.

5.4.4. Interaction effects of pond, year and season

Male BCI scores showed significant differences across year and season ($F_{12,540}$ = 2.65, p = 0.002) and year and pond ($F_{8,540}$ = 2.25, p = 0.02) but not pond and season ($F_{6,540}$ = 2.00, p = 0.06). This suggests that variation between years has the greatest effect on BCI, followed by season. The effects of inter-pond variation are the least. Due to lack of data in some months, a combined interaction of pond, year and season could not be performed.

Female BCI scores showed a similar pattern to that shown by males with a significant difference across year and season ($F_{4,402}$ = 2.90, p = 0.02) but not

pond and season ($F_{2,402}$ = 1.40, p = 0.25). This again suggests that variation between years has the greatest effect on BCI, followed by season with the effects of inter-pond variation the lowest. Due to lack of data a pond versus year and a combined interaction of pond, year and season could not be performed.

5.4.5. Effects of winter temperature & rainfall

There was a slight negative (non-significant) relationship between mean winter temperature each year and mean male body condition index scores the following spring (Figure 5.8) (R^2 = 0.06, F = 0.19, p = 0.69). Therefore body condition in spring was not affected by winter temperature over the five years duration of the study. There was a slight positive (non-significant) relationship between mean minimum December to February air temperature and mean female body condition the following spring (Figure 5.8) (R^2 = 0.43, F = 2.27, p = 0.23). Although winter temperatures appear to affect females differently to males, more data is required to determine whether these relationships persist over a longer time period.

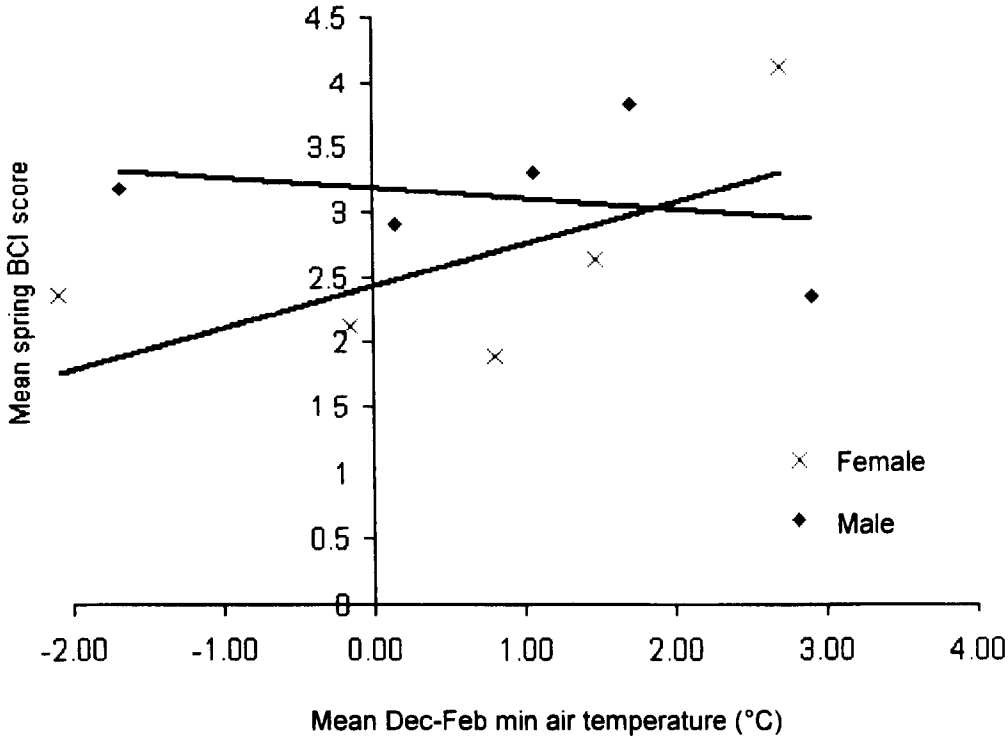


Figure 5.8. Multiple linear regression to show the effect of mean December to February air temperature on mean male and female body condition index (BCI) scores.

There was a slight negative (non-significant) relationship between non-aquatic rainfall (November to February) and mean male BCI scores (Figure 5.9) (R^2 = 0.21, F = 0.83, p = 0.43). This indicates that male BCI scores may be lower after wetter winters but more data is required to confirm this trend.

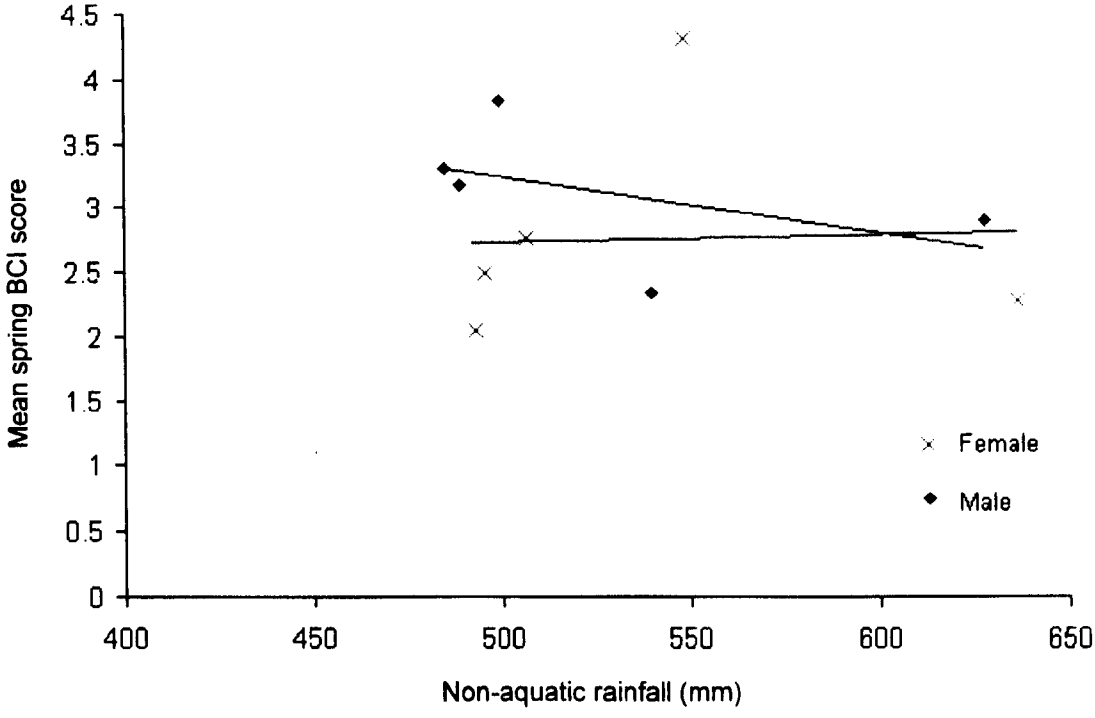


Figure 5.9. Multiple linear regression to show the effect of total non-aquatic rainfall, November to February, on mean male and female body condition index (BCI) scores.

There was also no significant relationship between total non-aquatic rainfall and mean female body condition index scores (Figure 5.9) (R^2 = 0.001, F = 0.004, p = 0.95).

5.5. Discussion

5.5.1. Differences in body condition between ponds

Results from this five-year study show that neither males nor females showed a significant difference in body condition between ponds. Analysis of the interaction between different factors (years, seasons and pond) also indicates that variation between ponds had the least effect on BCI in both sexes. Other studies have shown that differences in biotic and abiotic conditions may differentially affect individuals within a metapopulation. For example Sztatecsay & Schabetsberger (2006) found that body condition in the common toad *Bufo bufo* varied between sites and this was due to variation in habitat conditions. Also, Reading (2010) noted that tadpoles growing in ponds with higher temperatures had higher BCI scores. This demonstrates the potential effects of different microhabitat conditions such as light, temperature and vegetation cover. However my findings suggest that although there are differences in microhabitat conditions between the ponds (as shown by differences in HSI scores, Chapter 3), these are not great enough to cause significant variations in BCI scores at breeding ponds.

5.5.2. Differences between years and seasons

Both males and females showed significant differences in body condition index between years with significant interaction between both years and seasons. However, only males showed a significant variation in BCI between seasons. The pattern of BCI scores across years was considerably different for each sex. Males had the lowest BCI (and thus fat reserves) in 2007, before scores rose in 2008 and levelled off in the period 2009 to 2011. In contrast females had the highest BCI scores (and thus fecundity) in 2007, followed by a drop in BCI

scores through the rest of the study period. The reasons for the observed differences in BCI between the sexes may be because BCI scores are likely to indicate fecundity in females and fat reserves in males. Individuals from different sexes are likely to be differentially affected by variations in biotic and abiotic factors in different years at breeding and overwintering habitats. In addition males and females exhibit different microhabitat utilisation in aquatic and terrestrial habitats, which may affect acquisition of fat reserves or development of eggs in males and females respectively.

Other studies on amphibians have shown that BCI scores vary over time (Arntzen et al., 1999; Wheeler et al., 2003; Băncillă et al., 2010) but few have documented differences in sexes between years. Griffiths & Mylotte (1987) state that male great crested newts probably do not feed on the way to breeding ponds, however they do appear to take prey on arrival at the aquatic environment (Griffiths & Mylotte, 1988). Griffiths (1986) recorded differences in prey captured by different co-habiting newt species but not between male and female great crested newts. Larger individuals are able to capture larger prey (Griffiths & Mylotte, 1987) and because females are usually bigger than males (Andreone & Giacoma, 1989; Malmgren & Thollesson, 1999), this may lead to differences in prey capture by the sexes. Variations in prey availability in different years may therefore affect BCI in the sexes in slightly different ways. Further research specifically examining how biotic factors affect BCI scores is required before more definite conclusions can be drawn.

Male, but not female, great crested newts exhibited a significant change in body condition between seasons. Males showed low body condition at the start of the

season in the period 2007 to 2009, followed by a rise until May within each of these years. This pattern was not as evident in 2010 to 2011, which may be due to the small sample size. Body condition declined in June in three of the five study years. This trend appears contrary to that found by Arntzen et al. (1999) who found that great crested newts had highest BCI scores at the start of the year. Similarly Băncillă et al. (2010) noted that yellow-bellied toads had highest body condition index scores in spring and lowest in the autumn. However Verrell et al. (1986) observed a decrease in body fat content in smooth newts over the winter period. The reason why males had low BCI scores at the start of the season in my study may reflect specific microhabitat conditions in terrestrial habitats or differences in timings in emerging from hibernation and first breeding. Males may emerge from hibernation early with a low BCI score and require feeding in ponds to raise their body condition. Males in my study exhibited a drop in BCI score in June in three years. Arntzen et al. (1999) state this is due to high energy expenditure during the aquatic reproductive phase which lowers mass and therefore body condition.

Although differences were non-significant, females exhibited the opposite trend to males with a decreasing BCI score through the breeding season in all years except 2009. A high BCI score at the start of the season is expected due to females holding large numbers of eggs (Halliday & Tejedo, 1995). These will be lost through the breeding season, resulting in a drop of BCI score. However I restricted analysis to the early and mid parts of the breeding season (March to April) when females are still laying eggs. The observed drop in BCI score may be in part due to egg losses through oviposition, but may also reflect a decrease in fat reserves through lack of feeding and physiological stress of breeding.

However further data are required to determine whether this change is significant over a greater number of years.

5.5.3. Effects of winter temperature & rainfall

The effects of winter temperature and rainfall revealed non-significant trends in both males and females. Therefore further data are required to ascertain whether the observed trends become significant over a longer time period. The results highlight that long-term studies are required before significant inferences can be drawn from climatic data and its influence on body condition and survival.

5.5.4. Implications of findings

A variety of biotic and abiotic factors may play a role in affecting body condition and fecundity in male and female great crested newts respectively. These may include: microhabitat conditions within ponds such as degree of macrophyte cover; levels of inter- and intra-specific competition; terrestrial microhabitat availability; variations in winter conditions. Therefore management seeking to maintain adults in high body condition should aim at providing ideal terrestrial habitats and breeding ponds to provide conditions suitable for enhancing BCI scores. These include the SI factors identified by Oldham et al. (2000) in the great crested newt habitat suitability index.

5.5.5. Critique of methods

1. BCI scores for each sex in spring were estimated for individuals over a month. Individuals arriving at ponds at either the beginning or end of that time period may have had different BCI scores. Drift fencing and pitfall trapping may

have captured a greater proportion of individuals migrating to each pond. However due to logistical, time and access issues, installing a drift fence was not possible.

2. It was difficult to make any clear link between BCI scores and one microhabitat variable in these ponds due to the many factors present simultaneously. Laboratory or mesocosm experiments may be performed to enable more effective isolation of factors e.g. temperature, competition or predation, to determine their effect on BCI scores.

5.6. Conclusions

1. Neither males nor females exhibited significant variation in BCI scores between ponds.

2. Body condition index scores varied significantly between years in both sexes. Years with highest and lowest BCI scores were different for males and females possibly because BCI indicates fecundity in females and fat reserves in males.

3. Males, but not females, exhibited significant differences in BCI scores between seasons. Males tended to have low BCI scores at the start of the season before levels rose and peaked in May.

4. There was no significant effect of winter temperature and rainfall on ether male or female great crested newts. Studies over a greater time period are required to determine significant in trends.

Chapter 6. The effects of body condition on crest height in male great crested newts *Triturus cristatus*.

6.1. Abstract

Males of many amphibian, bird and mammal species produce secondary sexual characters prior to breeding to attract females. Several hypotheses have been posed to explain the existence for such features, including the 'good genes' theory which states that males with larger or more attractive features can withstand predation and disease, and may be honest indicators of fitness. Male great crested newts produce dorsal crests during the field season to use in courtship displays and individuals with larger crests secure more mates. Analysis of male great crested newt crest height across five breeding seasons March to June revealed a significant positive relationship between male crest height and snout-vent length as well as body condition, indicating that larger males and those with a higher body condition possessed a higher dorsal crest. The relationship was strongest in March and least in June, suggesting that body condition may affect crest height at the beginning of the breeding season when males are developing crests. Crest height in male great crested newts may therefore be an honest indicator of fitness but further research is required to determine the extent to which females actively choose males with larger crests.

6.2. Introduction

Darwin (1871) first proposed the theory of sexual selection which can be defined as 'a process which results in differential mating success among individuals within a population' (Panhuis et al., 2001). Darwin (1871) stated that this occurred either by competition within one sex for members of the opposite sex or differential choice by members of one sex for members of the opposite sex. The former often leads to male-male competition and can favour the evolution of traits such as those related to fighting ability which increases success in direct competition with other males for a mate. Alternatively, the latter (often known as female choice) can lead to the evolution of characteristics such as those involved in courtship displays which may result in males being more attractive to females (Kodric-Brown & Brown, 1984). Males of many animal species may develop exaggerated characteristics such as larger body size (Serrano-Meneses, 2006), weapons (e.g. enlarged chelipeds in crustaceans (Yoshino et al., 2011), antlers in deer (Hoem et al., 2007)), or develop bright colouration (Dijkstra et al., 2009). The exact mechanism behind the evolution of such features remains an area of much debate (Anderson, 1994; Kodric-Brown & Brown, 1984). There are three main hypotheses. The first is Fisher's (1930) runaway selection model, which states that females prefer males with an exaggerated feature which has an initial survival advantage. By selecting males with the largest trait, over many generations such features may enlarge as a result of directional selection to the point at which it has a negative effect on survival. The second is Zahavi's (1975) handicap hypothesis whereby an exaggerated trait demonstrates that males can survive despite the handicap of this feature. Third, is the truth in advertising model (Kodric-Brown & Brown, 1984) or 'good genes' theory (Halliday, 1978; Trivers, 1972) which proposes

that selected traits are costly to produce and maintain and this is correlated with phenotypic vigour. Males with a larger trait are thus likely to have increased growth, predator avoidance, disease avoidance and competitive ability (Kodric-Brown & Brown, 1984). An alternative form of this theory is the Hamilton-Zuk hypothesis, (Hamilton & Zuk, 1982) where traits are honest indicators of the ability to survive parasite or pathogen burdens. Males with a higher genetic vigour and thus larger trait are supposed to have lower parasite burdens. Over recent decades much controversy has existed in the scientific literature, especially in relation to the genetics of the 'good genes' model. Geneticist's state, particularly in relation to lekking species, where males gather to display and females only gain sperm, that directional selection will degrade the genetic variation for secondary sexual traits so that females will no longer gain from discriminating among males based on these traits (Miller & Moore, 2007). This has resulted in further refinements to the good genes model such as the 'good parenting genes' hypothesis, whereby a female choosing to mate with an elaborate male may produce daughters with 'good parenting genes' (Miller & Moore, 2007).

Recent research has focussed on finding examples of elaborated features as a result of 'good genes' where by males with larger, brighter or more complex secondary sex characters may be regarded by females as having higher fitness and reproductive potential. Examples include anurans (Forsman & Hagman, 2006), insects (Bailey & Zuk, 2008; Hoefler et al., 2008), fish (Dijkstra et al., 2009; O'Steen et al., 2010), mammals (Clutton-Brock, 2007; Vanpé et al., 2007) as well as numerous bird species (Loyau et al., 2007; Møller & Alatalo, 1999). However there still remains a debate as to the relative contribution of the 'good

genes' theory. Møller & Alatalo's (1999) review article found that there was a small correlation between offspring survival and expression of male secondary sex characters in a number of different species.

Within the order Amphibia, development of secondary sexual characters during the breeding season is widespread (Duellman & Trueb, 1986). In many species of anurans males use calls to defend territories and attract females (e.g. Bernal et al., 2009; Penna & Velásquez, 2010) and in several European species males may develop alternative or brighter colouration during the breeding season. For example male Balkan moor frogs *Rana arvalis wolterstorffi* turn blue (Ries et al., 2008) and European tree frogs develop colourful vocal sacs (Gomez et al., 2009). Males may also develop nuptial pads on their front toes before mating which facilitates attachment to females prior to external fertilization (Greene & Funk, 2009; Matthew & Miaud, 2007). Urodeles rarely engage in direct combat but females may choose mates based on development of secondary sexual characters as well as body size, age or prior sexual experience (Sullivan et al., 1995). Secondary sexual characters commonly take the form of brighter colouration (e.g. development of red ventral surface in the alpine newt *Mesotriton alpestris*) or growth of other features such as webbing on the feet (e.g. palmate newt *Lissotriton vulgaris*, Griffiths, 1996). Males of the *Triturus*, *Mesotriton* and *Lissotriton* genera perform a complex mating display to attract females (Hedlund, 1990) which involve fanning tails and pheromones towards females (Halliday & Tejedo, 1995). Many newts in Europe possess dorsal crests (e.g. great crested *Triturus cristatus*, Italian crested *T. carnifex* and smooth newts *Lissotriton vulgaris*) and these are thought to maintain a blood supply and act as an additional respiratory surface (Griffiths & Mylotte, 1988). This may

allow males to remain longer underwater without rising for air and allow more prolonged periods of courtship display (Griffiths & Mylotte, 1988). Previous research on great crested newts also indicates that crest height changes through the season with an increase in crest height through the early part of the season (Verrell & Halliday, 1985). However once males finish breeding, crest height then diminishes (Griffiths & Mylotte, 1988).

Male great crested newts with a longer snout-vent length have larger crests (Baker & Halliday, 2000; Hedlund, 1990) and in one study these males had greater mating success (Green, 1991). In the related smooth newts *Lissotriton vulgaris* females actively choose to mate with males with larger dorsal crests (Gabor & Halliday, 1997). In addition Gabor et al. (2000) observed higher mating success in male red spotted newts *Notophthalamus viridescens* which had a larger tail depth. Since males with larger crests may be able to absorb more oxygen from the water, this may allow males with larger crests to continue in courtship display for a longer time period before having to rise to the surface for air. Alternatively, the crest may confer additional information to females. Baker (1992) measured tail depth (which correlates with crest height) on great crested newts before they entered the breeding ponds and found a positive correlation with body condition. This suggests that males with higher body condition prior to breeding are able to develop larger crests, possibly because they have more resources available for growth of this secondary sexual characteristic. Therefore crest height may be an honest indicator of phenotypic vigour and recent foraging intake (Green, 1991). However Baker's (1992) study was only carried out in one population over one field season. Therefore in this study I examined the crest height and body condition of great crested newts

from four ponds over five years to determine whether the work by Baker (1992) was applicable to a greater number of ponds over a longer time span. I had the following aims:

1. To determine whether there was a significant relationship between male body condition and crest height in between years, seasons and ponds.

2. To assess the changes in male crest height across the breeding season.

6.3. Methods

6.3.1. Sampling methodology

Male great crested newts were captured from three breeding ponds over a five-year period as described in Chapter 2. Crest height is often difficult to measure in the field but tail depth correlates strongly with crest height and is easily and accurately measured (Griffiths & Mylotte, 1988). Therefore tail depth was measured as an index for crest height. Tail depth was measured using a plastic ruler to the nearest 0.5 mm. Analysis of Variance (ANOVA) was used to determine whether there were any differences in male crest height across months of the breeding season.

6.3.2. Data analysis

Body condition index (BCI) scores were calculated using the residuals method as described in Chapter 5. Multiple regression analysis was used to examine the relationship between crest height, BCI and snout-vent length (SVL) across all years and ponds. Linear regression was used to examine the relationship between crest height and body condition across years, months and ponds.

6.4. Results

6.4.1. Relationship between SVL, body condition and crest height

There was a significant positive relationship between male tail depth and snout-vent length across all years and ponds (Figure 6.1a) (R^2 = 0.20, F = 9.21, p = 0.0025). Males with a larger body length are therefore more likely to possess higher crests. There was also a significant relationship between male tail depth and body condition index (Figure 6.1b) (R^2 = 0.14, F = 88.11, p < 0.0001). This indicates that males with a higher body condition index score are more likely to have a higher crest height.

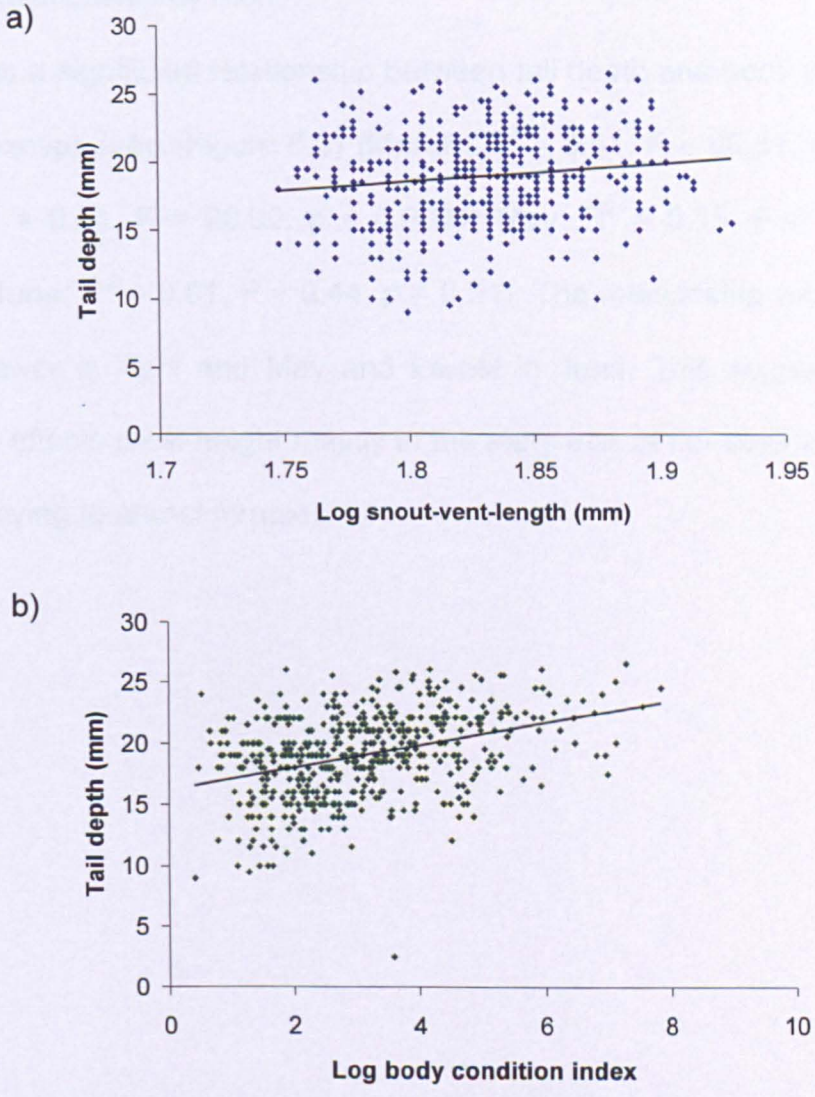


Figure 6.1. Multiple regression of a) male tail depth against snout-vent length; b) male tail depth against body condition index.

6.4.2. Relationship between crest height and body condition

6.4.2.1. Relationship by year

There was a significant relationship between tail depth and body condition in all years except 2007 (Figure 6.2) (**2007**: $R^2 = 0.003$, $F = 0.47$, $p = 0.69$; **2008**: $R^2 = 0.15$, $F = 34.74$, $p < 0.0001$; **2009**: $R^2 = 0.29$, $F = 28.91$, $p < 0.0001$; **2010**: $R^2 = 0.48$, $F = 53.03$, $p < 0.0001$; **2011**: $R^2 = 0.15$, $F = 4.36$, $p = 0.04$). This indicates that males with a higher body condition tend to have a larger crest height across multiple years.

6.4.2.2. Relationship by month

There was a significant relationship between tail depth and body condition in all months except June (Figure 6.3) (**March**: $R^2 = 0.51$, $F = 60.51$, $p = < 0.0001$; **April**: $R^2 = 0.11$, $F = 28.90$, $p < 0.0001$; **May**: $R^2 = 0.15$, $F = 32.55$, $p = < 0.0001$; **June**: $R^2 = 0.01$, $F = 0.44$, $p = 0.51$). The relationship was strongest in March, lower in April and May and lowest in June. This suggests that body condition affects crest height mainly in the early part of the season when males are displaying to attract females.

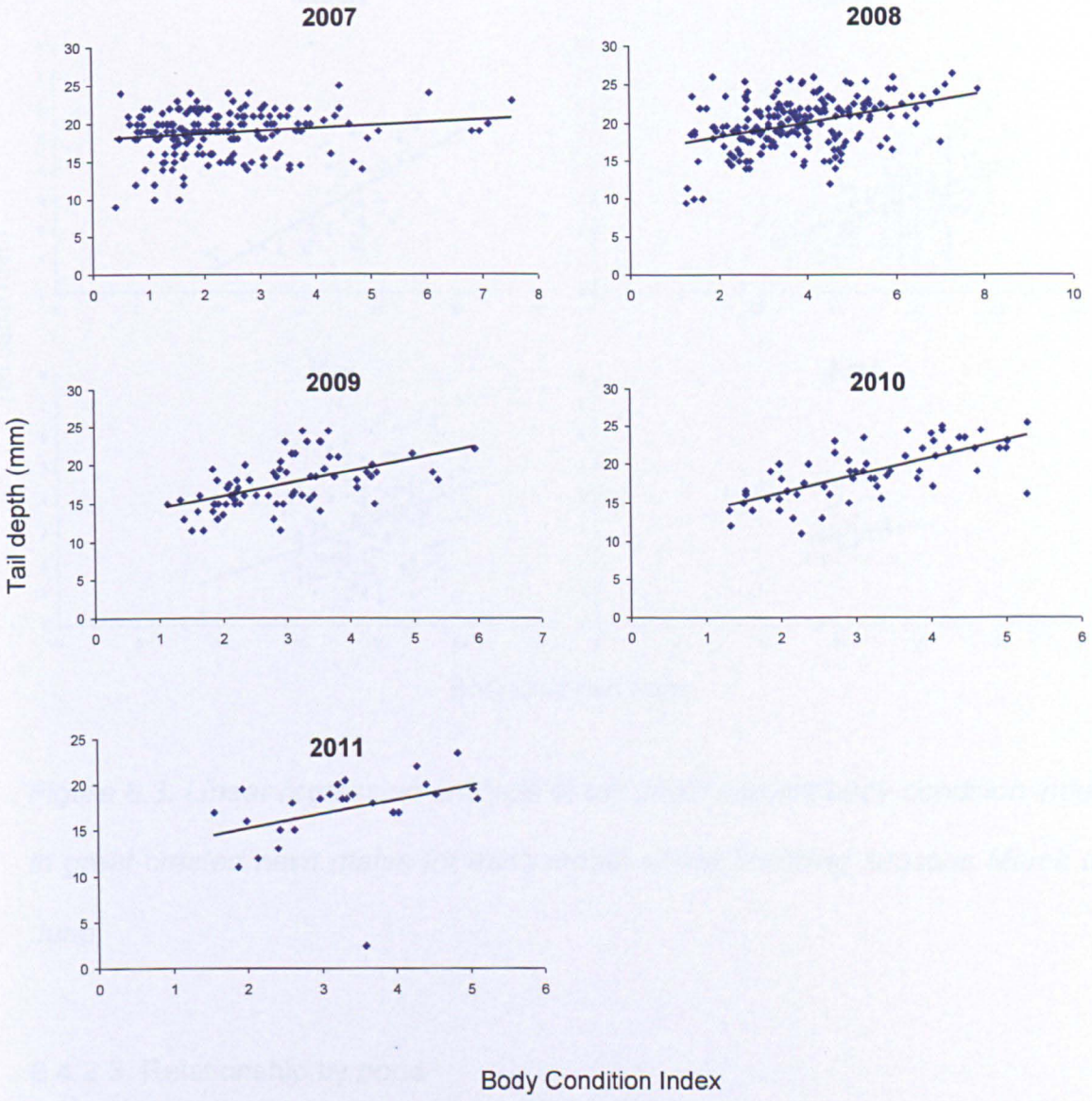


Figure 6.2. Linear regression analysis of tail depth against body condition index in male great crested newt for each year, 2007 to 2011.

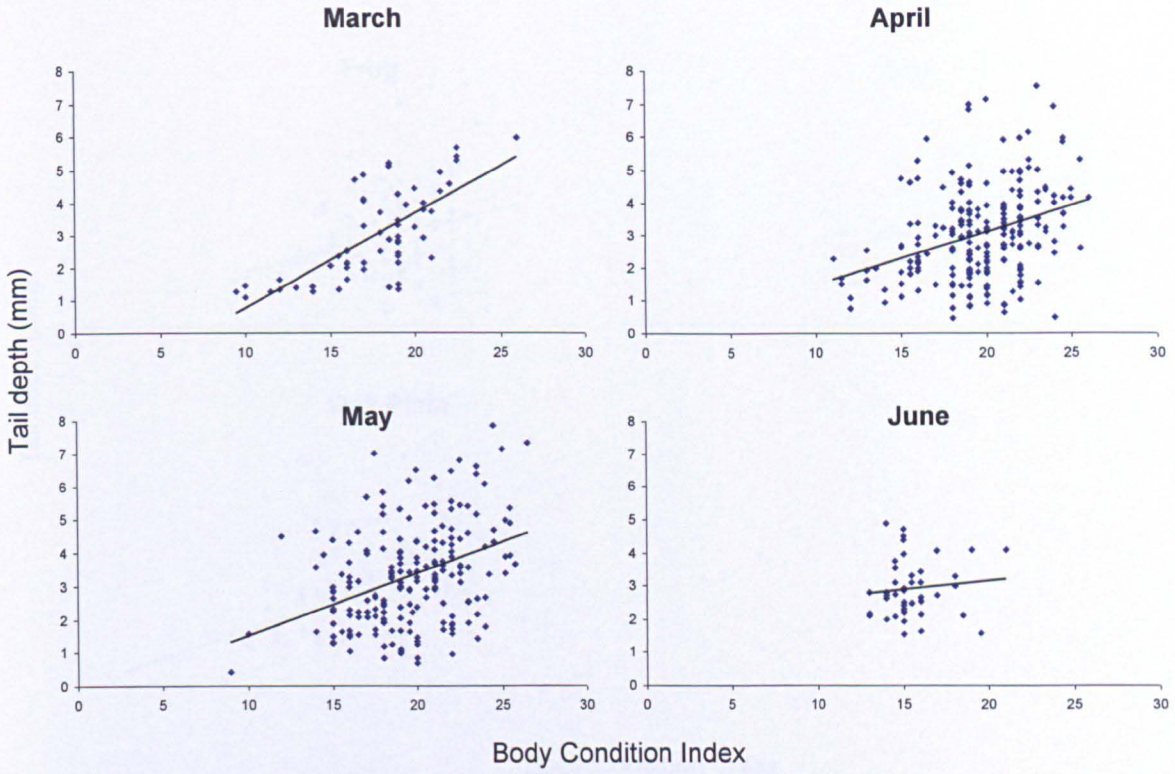


Figure 6.3. Linear regression analysis of tail depth against body condition index in great crested newt males for each month of the breeding seasons March to June.

6.4.2.3. Relationship by pond

There was a significant relationship between tail depth and body condition in all three ponds (Figure 6.4) (**Frog**: $R^2 = 0.09$, $F = 20.86$, $p = < 0.0001$; **Lily**: $R^2 = 0.19$, $F = 13.26$, $p < 0.001$; **Oak Plain**: $R^2 = 0.17$, $F = 56.80$, $p = < 0.0001$). This indicates that the positive relationship between tail depth and body condition is found in more than one population. However the amount of variation in tail depth that was explained by body condition was low in all months (range: 9 to 19%). This suggests that although body condition may explain some variation in crest height among males, the amount is likely to be low.

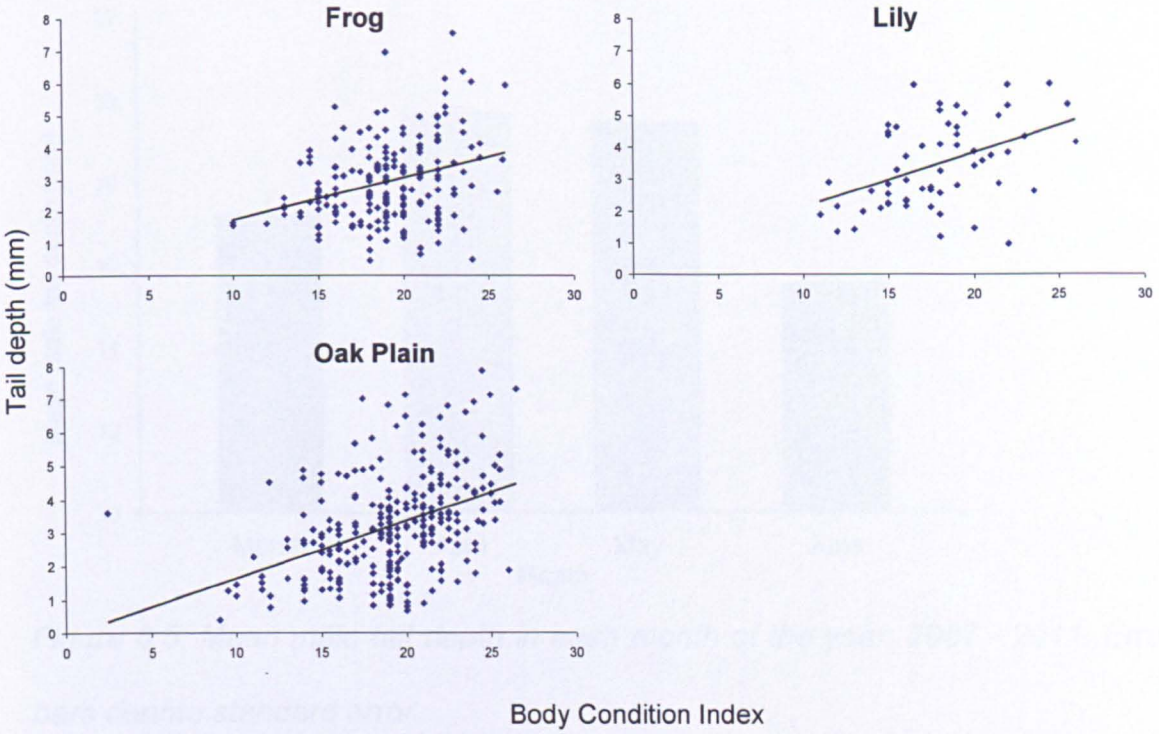


Figure 6.4. Linear regression analysis of tail depth against body condition index in great crested newt males for each pond.

6.4.3. Crest height morphology between seasons

There was a highly significant difference in tail depth and hence male crest height between seasons in all years (Figure 6.4) ($F_{3,546}$ = 33.44, $p < 0.0001$). Male tail depth started at a fairly low level (mean 17.2 mm) in March before rising through the early part of the breeding season to peak at 19.65 in April. Tail depth values remained high in May and declined rapidly in size to (15.5) in June at the end of the breeding season.

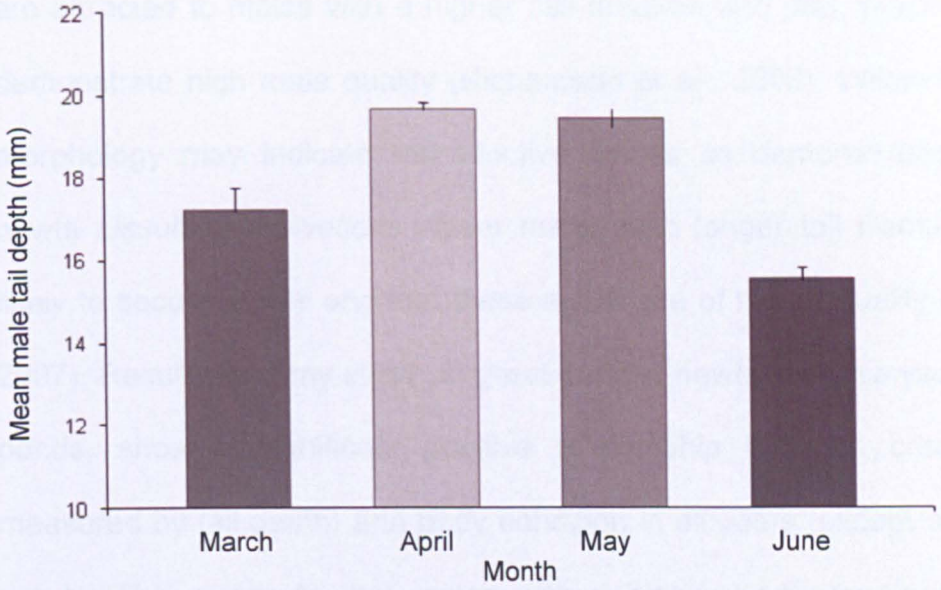


Figure 6.5. Mean male tail depth in each month of the year, 2007 - 2011. Error bars denote standard error.

6.5. Discussion

6.5.1. Relationship between SVL and crest height

Previous studies have demonstrated a positive relationship between crest height and SVL in great crested newts (Baker & Halliday, 2000). My results also show the same relationship although this was only observed in 6% of the population. There appears to be a large variation in crest height for a given SVL, probably because crest height diminishes as the season progresses (Griffiths & Mylotte, 1988).

6.5.2. Relationship between body condition and crest height

Secondary sexual characters as honest indicators of fitness have been demonstrated in many anuran species which use calls to attract mates. Female poison frogs *Dendrobates leucomelas* prefer to mate with males which have louder and more frequent calls and these 'good' callers produce higher quality offspring (Forsman & Hagman, 2006). European female treefrogs *Hyla arborea*

are attracted to males with a higher call duration and rate, which is thought to demonstrate high male quality (Richardson et al., 2008). Within the Urodeles, morphology may indicate reproductive fitness as demonstrated by palmate newts *Lissotriton helveticus* where males with longer tail filaments are more likely to secure mates and that these males are of higher quality (Haerty et al., 2007). Results from my study on great crested newts over five years from three ponds, show a significant positive relationship between crest height (as measured by tail depth) and body condition in all years (except one) and three ponds. This suggests that males with a higher body condition are able to develop larger crests and this occurs in several populations over successive years. Baker (1992) carried out similar research on great crested newts but this was in a single population over one breeding season. My study extends these findings to a greater number of years and ponds. In my study, the relationship between crest height and body condition was strongest in March, which is at the beginning of the season, when males are developing crests. Baker (1992) carried out his study as males arrived at their breeding pond and my results show this time period and soon after to be the most critical in determining crest height. As the season progresses, crest height drops (Griffiths & Mylotte, 1988), which may be due to males not requiring crests for display or depletion of food reserves.

Previous research indicates that larger crest height confers greater mating success in male great crested newts (Baker & Halliday, 2000; Hedlund, 1990, Malmgren & Enghag, 2008). If males with a high body condition at the start of the breeding season are more likely to have larger crests, this suggests that these individuals have more resources available. Therefore crest height at this

time of year may be an honest indicator of fitness in great crested newts. However no evidence has determined whether females actually select males with larger crests, or whether a larger crest favours greater mating success simply through an ability to display for longer periods without rising for air. However, the finding that males with a higher body condition have larger crests demonstrates the importance for males in maintaining body condition through the winter months. Recent research by Griffiths et al. (2010) has shown that male great crested newts are more likely to start the breeding season with a lower body condition after milder winters. If the trend for warmer winter temperatures continues this may have significance for an individual male's ability to maintain body condition over the winter and thus grow a large crest in spring. This in turn may affect his reproductive success.

6.5.3. Crest height morphology between seasons

In this study, crest height at the beginning of the season was relatively low. This probably reflects the immigration of males to ponds at a time when only some individuals will have developed full crest height. Previous studies indicate that in great crested newts, crests only develop once males reach breeding ponds (Griffiths & Mylotte, 1988; Verrell & Halliday, 1985). This was supported in my study by a rapid increase in crest height once males had arrived at breeding ponds. By April, the majority of males would have arrived at ponds (Verrell, 1987) and had sufficient opportunities to form crests. In June crest height fell quickly to the lowest value in the season. At the end of the breeding season males no longer require their crests for breeding and therefore these characteristics regress (Griffiths & Mylotte, 1988). Therefore results from my

study support those of others with male crest height varying with month and following a typical pattern in all years.

6.5.4. Critique of methods

1. Body mass in 2007 was measured using hand-held spring Pesola scales which proved inaccurate due to the wind blowing the bag containing each newt. Therefore in the remaining years, electronic digital scales were used which gave more accurate body mass readings and thus BCI scores.

2. Values for SVL were difficult to obtain in the field without anaesthetising individuals. This was because newts tended to squirm when handled and only remained still for a few seconds. This may have resulted in inaccurate measurements of SVL, especially at the start of the study (2007). This would have led to less accurate estimates of BCI scores. This may explain some of the large variations in standard error for these measurements.

3. Crest height was not measured directly. Although there is a strong relationship between tail depth and crest height, more accurate data would be obtained if crest height could have been measured.

6.6. Conclusions

1. There was a significant positive relationship between crest height, SVL and body condition index score in male great crested newts which was consistent across four years and three ponds. This adds to the evidence that males with a higher body condition are more likely to develop higher crests.

2. The relationship between crest height and body condition was most significant as the beginning of the breeding season in March. By June there was no relationship between these two variables.

3. Crest height declined through the breeding season, a finding similar to those of other studies.

Chapter 7. Non-consumptive effects of predatory three-spined sticklebacks *Gasterosteus aculeatus* on great crested newt *Triturus cristatus* embryos.

7.1. Abstract

Predatory fish have negative impacts on many amphibian populations, often through direct predation on embryos and larvae. The presence of predators during embryonic development may elicit adaptive responses in emerging larvae. This study examined the non-consumptive effects of predatory three-spined sticklebacks *Gasterosteus aculeatus* on great crested newt *Triturus cristatus* embryos under controlled conditions. Embryos raised in the presence of sticklebacks but in predation-proof enclosures suffered significantly higher mortality compared to control treatments in three independent trials over two years. Overall 26.9% of embryos hatched in stickleback treatments compared to 47.6% from controls. As sticklebacks were treated with fungicide before the experiments, this difference in mortality is unlikely to be due to fungal disease transmission. There were no significant differences in the date, stage of development and size at hatching in larvae raised with and without sticklebacks. Results suggest the potential for negative non-consumptive impacts of predatory sticklebacks on great crested newts during the embryonic stage.

7.2. Introduction

The direct predatory effects of fish on amphibians have been well documented in recent years (Beebee, 1996; Hecnar & M'Closkey, 1997; Knapp & Matthews, 2000; Joly et al., 2003). In Europe, introductions of fish to freshwater lakes have been one of the largest factors contributing to amphibian reproductive failure (Orizaola & Braña, 2006). Many amphibian eggs and larvae are often at particular risk due to their exposure to predators. Direct predation by predators on eggs and larvae can often drastically reduce numbers and in some cases eliminate whole populations (Brönmark & Edenhamn, 1994; Monello & Wright, 1999). However predators may also have negative indirect, or non-consumptive, effects on amphibian embryos. Several studies have demonstrated the potential for predatory fish to transfer fungal pathogens, notably *Saprolegnia* species, to amphibian embryos during development (Kiesecker & Blaustein, 1999; Kiesecker et al., 2001). When infected with *Saprolegnia* species some amphibian embryos appear to exhibit increased mortality, resulting in decreased hatching success (Blaustein et al., 1994a; Romansic et al., 2007; Fernández-Benéitez et al., 2008). There is therefore potential for fish to have non-consumptive negative impacts on amphibians at the embryonic stage.

The negative impacts of predation may be reduced if larvae exhibit adaptive responses such as decreased movements (Van Buskirk et al., 1997; Van Buskirk & McCollum, 2000; Relyea, 2002a) or morphological plasticity such as developing broader tails (Van Buskirk & Arioli, 2002; Relyea, 2004) when faced with predator chemical cues. These appear to reduce the effects of predation and thus increase larval survival. There is also increasing evidence to suggest

that amphibians can respond to predator chemical cues whilst in the embryonic stage. Embryos in immediate danger may exhibit a threat-sensitive response by hatching at an earlier date and stage of development (Saenz et al., 2003; Gomez-Mestre et al., 2008), thus allowing emerging larvae to escape predation risk. If larvae are at greater risk from predation, embryos may hatch at a later date and in a more advanced stage of development (Ireland et al., 2007; Mandrillon & Saglio, 2009). Larvae hatching from embryos which have developed in the presence of predator cues may also exhibit altered behaviour, such as increased hiding responses (Mathis et al., 2008; Ferrari & Chivers, 2009) or altered morphology (Ireland et al., 2007) such as emerging at a larger size for enhanced swimming. These responses decrease the chances of emerging larvae being caught by predators.

Great crested newts *Triturus cristatus* breed in permanent or semi-permanent water bodies that often support populations of fish. Their larvae appear particularly susceptible to predation by fish due to their nektonic behaviour (Joly et al., 2001) and whole populations can be eliminated by fish species (Beebee, 1997; Cooke, 1994). Although adults may avoid breeding in fish ponds using chemical cues (Malmgren, 2003), this may only apply to new immigrants (Beebee, 2007). Returning adults, that are highly faithful to breeding ponds, often still breed where fish are present.

Three-spined sticklebacks *Gasterosteus aculeatus* are small, predatory, fast-swimming fish which readily consume the larval stages of great crested newts (Oldham et al., 2000). In ponds with sticklebacks, populations of newts are often decimated (Cooke, 1994; English Nature, 2001; Beebee, 2007). Removal of

sticklebacks from ponds can result in recovery of great crested newt populations (McLee & Scaife, 1992) demonstrating the potentially negative effects of this species. Although it is observed that sticklebacks affect newt populations through direct predation, no study has examined the possibility of indirect, or non-consumptive, effects of sticklebacks on embryos before they hatch. Only 50% of great crested newt embryos are expected to survive to hatching due to a chromosome abnormality (Horner & MacGregor, 1985). Any negative effects on embryos will therefore have an even greater effect on the hatching success of this species. There are also no studies that have determined whether great crested newt embryos exhibit plasticity in development while in the presence of predators, which may reduce direct predation on hatching larvae.

This study examined the non-consumptive effects of stickleback predator cues on great crested newt embryo development under controlled conditions. The main aims were to: (1) see if great crested newt embryos showed any changes in mortality when developing in the presence of predators while protected from consumption; (2) determine whether great crested newt embryos exhibited plasticity in timing of development and morphology whilst developing in the presence of predatory sticklebacks.

7.3. Methods

Experiments were carried out in three independent trials: early and late season 2008 and late season 2009. Due to cold conditions experienced in the early part of 2009, no early season experiments were carried out. The 2009 experimental

trial had different methodology to eliminate the potential for fungal transfer from sticklebacks to embryos.

7.3.1. 2008 methods

Ten opaque plastic containers (55 cm L x 45 cm W x 33 cm D) were filled with aged tap water and placed on a laboratory bench at room temperature (20 °C ± 3 °C) and were subject to natural light:dark cycles. Egg strips, consisting of black plastic bags cut into strips approximately 2 cm wide and 50 cm long, were placed into a local great crested newt breeding pond in March 2008. After 48 hours, strips were removed from the pond and eggs counted. Eggs were unwrapped, to allow assessment of stage of development (staging table in Gallien & Bidaud, 1959), but remained on strips, and divided into equal groups. Females may lay several eggs per strip as well as laying on multiple strips (personal observation). Therefore eggs from different strips were randomly assorted before grouping. Each group, containing approximately fourteen eggs, was placed into a mesh bag (20 x 20 cm; mesh diameter < 0.5 mm). Each mesh bag was sealed and one placed into every container, ensuring eggs were submerged. Sticklebacks were placed into alternate containers; the remaining five containers were left as controls. This allowed for five sticklebacks and five control replicates. Sticklebacks were fed every 48 hours using commercial fish flakes (TetraFin®: containing mainly fish derivatives, cereals, algae, molluscs and crustaceans). Care was taken to ensure fish ate all food immediately and none was left to contaminate the water. Tanks were not oxygenated during development since the relatively large volume of water (81 litres per tank) was assumed to contain enough oxygen for the duration of the experiments. Water pH and NO_3^- / NO_2^- analysis was carried out weekly in each container. After two

weeks, containers were checked daily for hatching by newt embryos. Date of hatching was noted for each replicate which was between two to three weeks from the start of the experiment.

Once all surviving embryos had hatched, four weeks after the start of the experiment, all containers were emptied, cleaned and the experiment repeated with the same procedure in May 2008. Different individual sticklebacks were used and twenty - rather than 14 - great crested newt eggs were placed into each container. Embryos were collected from the same breeding pond, which contains over 200 breeding females (personal records based on capture-recapture studies) so it is unlikely the same females laid on strips for each trial.

7.3.2. 2009 methods

In 2009 experiments embryos were left wrapped to reduce fungal transfer (e.g. *Saprolegnia* species) from fish to embryos. To avoid the adding fish food to experimental tanks, sticklebacks were left in containers for 48 hours before being returned to a separate oxygenated feeding tank. Different sticklebacks were then placed in experimental tanks. Sticklebacks in the feeding tank were also treated with commercial antifungal fluid silver proteinate concentration 0.0151% w/w. This was pipette into the water in the feeding tank (1 ml per 9 litres of water) and reducing the probability of transferring fungi to embryos.

7.3.3. Data analysis

The percentage of embryos hatching in each trial was calculated along with the mean percentage for each treatment. A two-way ANOVA was applied to arcsine-square root transformed proportion data to test for a difference in the

mean proportion of embryos hatching in control compared to stickleback treatments and across trials. The mean number of days to hatching, stage of development and total length at hatching were calculated for each replicate and each tested using a two-way ANOVA to determine differences between treatments and trials.

7.4. Results

7.4.1. Embryo survival

In all trials a significantly fewer proportion of embryos hatched from stickleback compared to control treatments (ANOVA $F_{1,24}$ = 13.18, P = 0.0013). Overall 26.9% of embryos hatched in stickleback treatments compared to 47.6% from controls (Figure 7.1). There was no significant interaction in hatching between trials (ANOVA $F_{2,24}$ = 0.55, P = 0.59) indicating no effects of trials on the proportion hatching. In control containers from all trials the mean percentage hatching from each tank was close to the expected 50%, assuming half of embryos will possess the chromosome abnormality. In stickleback treatments the mean proportions of embryos hatching from each trial were 23%, 24% and 33% respectively.

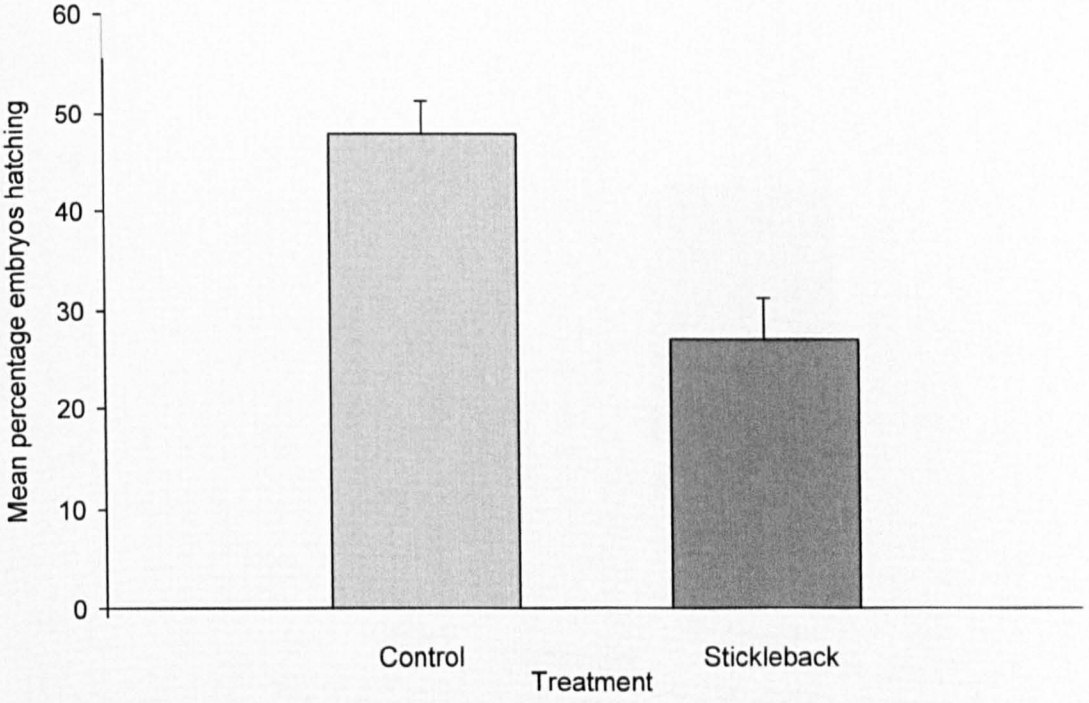


Figure 7.1. Mean percentage great crested newt embryos hatching in both stickleback and control treatments in three independent trials over two years. Error bars denote standard error. There is a signifiant difference between treatments, $F_{1,24} = 13.18$, $P = 0.0013$.

7.4.2. Date, stage of development and total length at hatching

There was no significant difference in the mean number of days to hatching, stage of development and total length at hatching of larvae hatching from stickleback compared to control treatments in all three trials (ANOVA $F_{1,24}$ = 1.33, P = 0.26; $F_{1,24}$ = 1.08, P = 0.31; $F_{1,24}$ = 1.34, P = 0.26 respectively (see Figures 7.2 to 7.4).

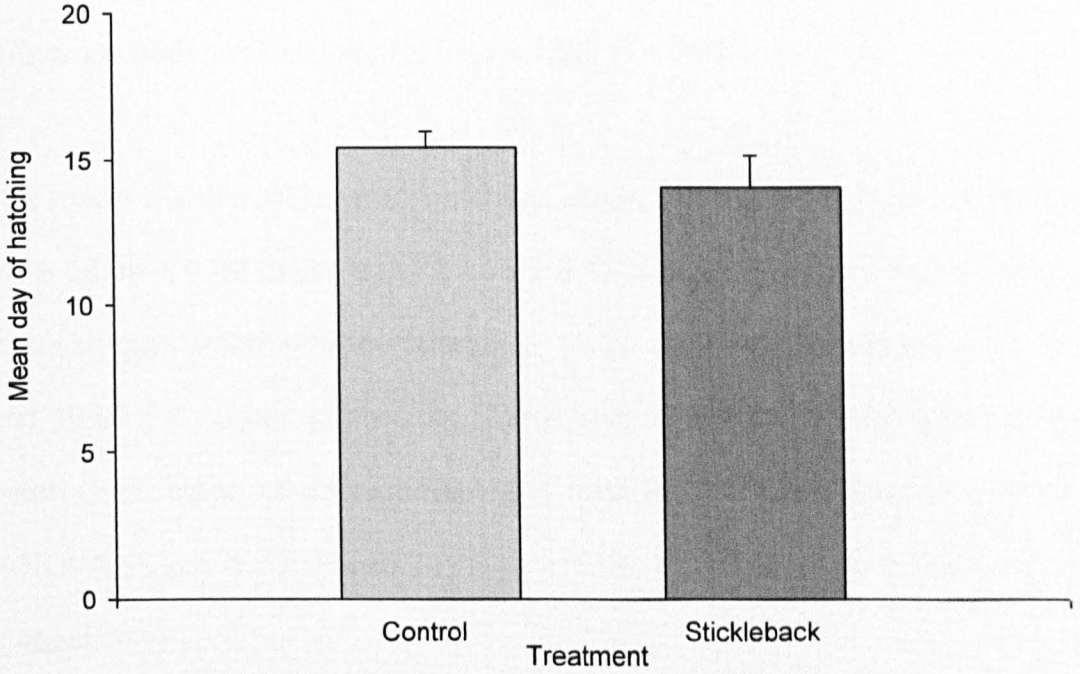


Figure 7.2. Mean day of hatching in great crested newt embryos in both stickleback and control treatments in three independent trials over two years. Error bars denote standard error. There is no significant difference between treatments, $F_{1,24} = 1.33$, $P = 0.26$.

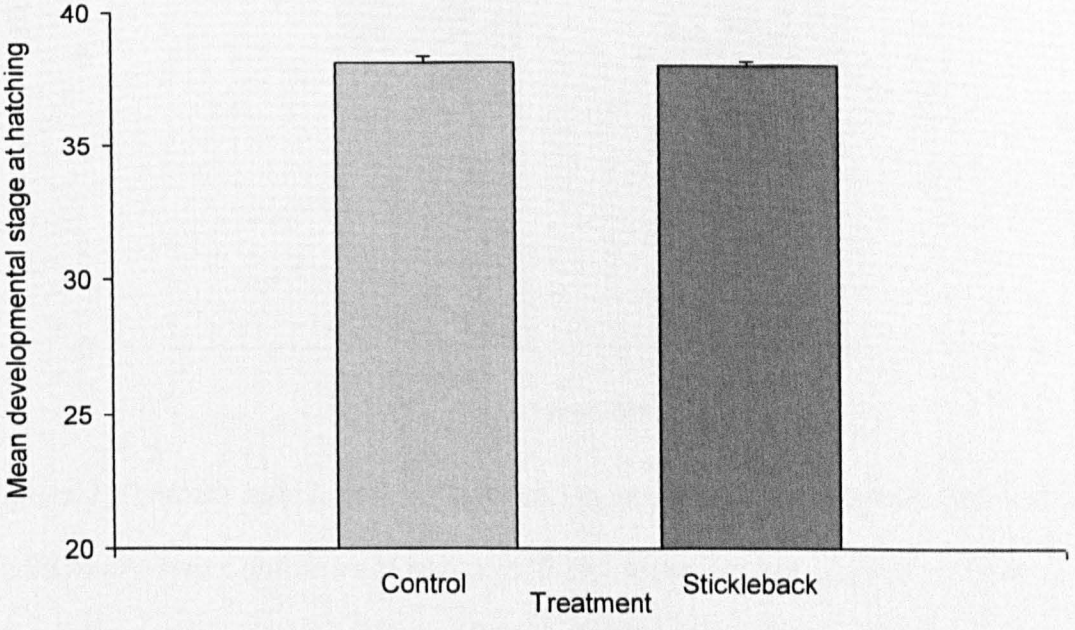


Figure 7.3. Mean developmental stage at hatching in great crested newt embryos in both stickleback and control treatments in three independent trials

over two years. Error bars denote standard error. There is no significant difference between treatments, $F_{1,24} = 1.08$, $P = 0.31$.

The mean number of days to hatching, stage of development and total length were 13.99 ± 0.49 days, stage 37.95 ± 0.19 and 10.79 ± 0.15 mm respectively in stickleback treatments compared to 15.34 ± 0.58 days, stage 38.13 ± 0.26 and 10.89 ± 0.13 mm in controls. There was no significant interaction between mean date, stage of development and total length at hatching between trials (ANOVA $F_{2,24} = 1.66$, $P = 0.21$; $F_{2,24} = 1.44$, $P = 0.34$; $F_{2,24} = 2.65$, $P = 0.09$ respectively).

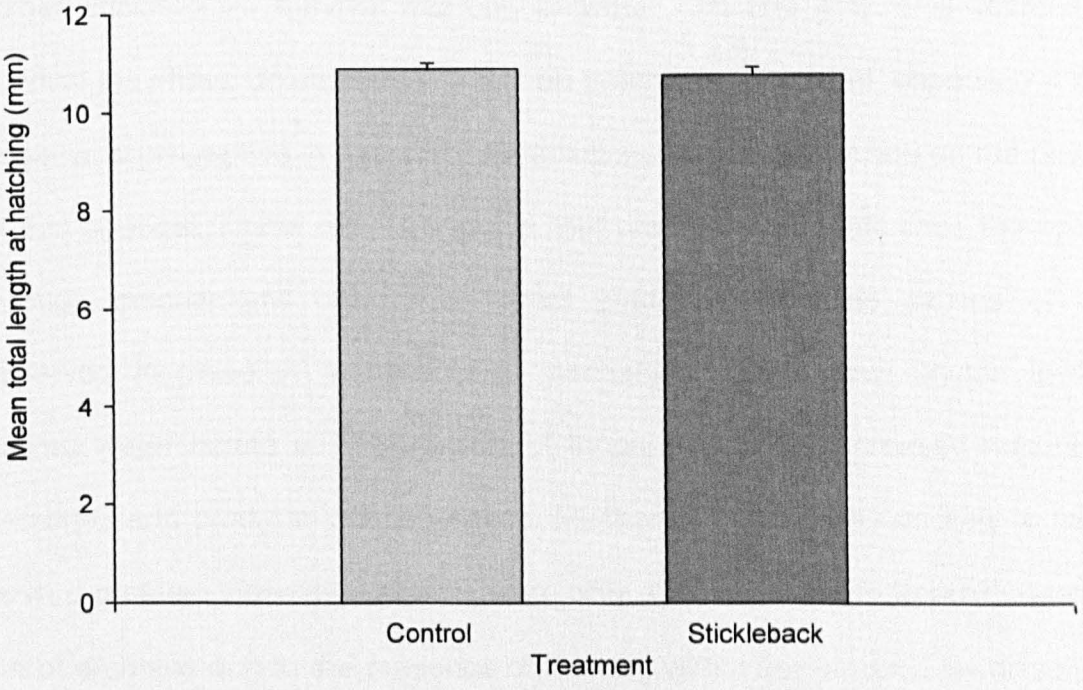


Figure 7.4. Mean total length at hatching in great crested newt embryos in both stickleback and control treatments in three independent trials over two years. Error bars denote standard error. There is no significant difference between treatments, $F_{1,24} = 1.34$, $P = 0.26$.

7.4.3. Water analysis

Water analysis revealed a constant pH of 7.0 in all containers in all trials. Water NO_3^- and NO_2^- never exceeded 25 mg/l and 1 mg/l respectively in all containers.

7.5. Discussion

7.5.1. Embryo survival

Results from this study show increased mortality of great crested newt embryos when developing in the presence of predatory stickleback cues. Great crested newts developing naturally lose 50% of embryos to a chromosome abnormality (Horner & MacGregor, 1985). However in this study, in the presence of three-spined sticklebacks, survival was only between 23% and 33%. This decreased survival may have deleterious impacts on potential recruitment, especially if the negative consumptive impacts of sticklebacks continue to operate on the larvae of this species. These results suggest that predators may influence their prey through mechanisms other than direct predation. Possible causes of the observed increase in mortality are: mechanical stress, low oxygen levels, altered water nitrate or pH, transfer of fungal infections, increased metabolic demands and predator-induced stress. Mechanical stress was unlikely to have been a problem since sticklebacks were only able to get within approximately 5 cm of embryos due to the presence of the bag with a fine mesh. Low dissolved oxygen may lead to abnormalities or asphyxiation (Olivier & Moon, 2010), especially in artificial conditions (Seymour & Bradford, 1995) but low oxygen levels are unlikely to have affected embryo survival since sticklebacks are small fish with low oxygen demands and would have been unlikely to decrease levels of oxygen in the relatively large containers over a short time period. Nitrogen

ions may have mixed effects on amphibians (Ortiz et al., 2004; Griffis-Kyle & Ritchie, 2007; Meredith & Whiteman, 2008), although many studies show deformities and increased mortality in the presence of these ions. Excretory products from fish may increase nitrogen ions to toxic levels (Mandrillon & Saglio, 2007). Water analysis in this study revealed that NO_3^- and NO_2^- did not exceed local pond water levels of 25 g/l and 1 mg/l respectively. It is therefore unlikely to have caused the increase in mortality. Altered pH is known to cause deleterious effects on amphibian embryos and larvae (e.g. Beattie et al., 1991). The presence of fish may alter water pH through the presence of excretory products or respiration. However the pH of the water in all containers remained consistently at 7.0 throughout the experiments, indicating altered pH is unlikely to have increased mortality in this species (Griffiths, 1993). The transfer of pathogens, especially the water mould *Saprolegnia,* from predatory fish to amphibian embryos has been demonstrated in a number of cases (Kiesecker et al., 2001, Kiesecker & Blaustein, 1999, Fernández-Benéitez et al., 2008). Results from this study show transmission of fungal infections from sticklebacks to developing great crested newt embryos was unlikely since sticklebacks were treated with antifungal fluid in the last two trials and there were no significant differences in survival between these and the first trial where fish were untreated. Fish are known to produce chemicals, or kairomones, from their skin surface (Ślusarczyk, 1999; Lass & Bittner, 2002; Lass et al., 2005). These are either produced from the skin mucus cells or from bacteria which inhabit skin surface (Ringelberg & Van Gool, 1998, in Weber, 2003). The effects of fish kairomones have been found to induce adaptive responses in amphibians (Relyea & Mills, 2001; Relyea, 2003). It is possible that stickleback kairomones affect newt embryos by inducing a stress or metabolic response, which may

affect immune function. This may lead to increased susceptibility to water-borne pathogens, including bacterial and fungal diseases. Few experimental studies have demonstrated increased amphibian embryo mortality induced by fish kairomones but in a study of damselfly larvae *Lestes viridis*, Slos et al. (2009) found individuals experienced increased mortality when in the presence of fish kairomones due to the stimulation of the fight or fright response, leading to oxidative stress by individuals. Therefore a suggested reason for the increased mortality of great crested newt embryos in the presence of sticklebacks as observed in these experiments is a result of a stress or metabolic induced response triggered by fish kairomones. Such a response may impair immune function, leading to increase in infection and subsequent mortality. However my experiments did not test this directly and determination of the actual causes of mortality remain for further investigation.

7.5.2. Date, stage of development and total length at hatching

Results from this study show no significant difference in the date, stage or length at hatching of great crested newt larvae in stickleback compared to control treatments. Although embryos of several amphibian species show plasticity in development and morphology when developing in the presence of predators (Mandrillon & Saglio, 2007; Mathis et al., 2008; Ferrari & Chivers, 2009), it appears such plasticity may only occur in certain species and under particular circumstances. For example, embryos of the common frog *Rana temporaria* appear to only alter development when faced with multiple stressors (Mandrillon & Saglio, 2009). Plasticity may vary depending on the predator and prey species involved. Embryos of the red-eyed tree frog *Agalychnis callidryas* respond to predators by emerging from eggs at an earlier stage of development,

while those of the gliding tree frog *A. spurrelli*, remain motionless. Also Ireland et al. (2007) found that tadpoles of the green frog *Rana clamitans* hatched at a smaller size and earlier stage of development when in the presence of leeches *Nephelopsis obscura* but a larger size and later stage of development when raised in presence of dragonfly nymphs *Aeshna candensis* (Mathis et al., 2008). Such phenotypic plasticity in development and morphology are costly (DeWitt et al., 1998) so larvae must experience relatively high predation risk to make plasticity worthwhile (Teplitsky et al., 2003; see also review in Bernard, 2004). For example Van Buskirk (2002) found larval *R. temporaria* did not alter morphology when raised with predatory dragonfly nymphs *A. cyanea* because the costs of altering body form did not outweigh predation risk. Similarly, Anderson & Petranka (2003) found that neither *R. sylvatica* nor *Ambystoma maculatum* showed any alterations in hatching time or stage of development when raised in the presence of predatory dragonfly nymphs. Again, costs in plasticity outweighing benefits gained were suggested as reasons for lack of response. In my study on great crested newts the degree of predation risk may be too low relative to the potential costs associated with altering hatching time or morphology. Embryos may have needed to face a greater predation risk to elicit a response. However further studies, with such increased predation risk, will be required to see if great crested newt embryos do exhibit an adaptive plasticity during development.

7.5.3. Critique of methods

There were a few limitations in the study design which were:

1. In 2008 trials, fish were fed in experimental tanks. The addition of extra substances to the tank may have affected water quality/composition. However

food levels were kept to a minimum and no effects on water pH or nitrogen were noticed.

2. During 2008 methods, embryos were unwrapped to allow accurate counting of eggs. This may have damaged embryos or increased the likelihood of acquiring a fungal infection. However because all eggs in this cohort were treated uniformly, this would not bias results. In addition, embryos in 2009 remained wrapped and the same results were obtained, demonstrating unwrapping of eggs did not adversely affect the experiment.

3. In 2009 fish were left in experimental tanks for 48 hours before being returned to a feeding tank. Whilst not in the experiment fish were housed in the same container which increased the risk of individuals picking up infection. To reduce this risk commercial antifungal fluid silver proteinate was used to treat fish and no evidence of infections was observed on sticklebacks.

7.6. Conclusions

Results from this study show increased mortality in great crested newt embryos when raised in the presence of predatory sticklebacks compared to controls. Further research is required to determine the reasons for the observed increases in mortality. There was no significant difference in date, stage of development and length at hatching in great crested newt larvae developing as embryos in the presence of predatory sticklebacks compared to controls. Lack of response may be because the degree of predation risk was too low to elicit developmental or morphological plasticity. Further research is required to determine whether increased predator presence elicits adaptive responses in

hatching larvae in this species. Overall, results suggest the potential for negative, non-consumptive effects of predatory fish on amphibian embryos under controlled conditions.

Chapter 8. Anti-predator responses of great crested newt *Triturus cristatus* larvae in the presence of caged fish.

8.1. Abstract

Predation by fish can have detrimental effects on amphibian populations through consumption of aquatic larvae. Larval amphibians may reduce mortality by exhibiting behavioural plasticity in microhabitat use and degree of movement. This study examined whether great crested newt larvae exhibit anti-predator responses when faced with either caged non-predatory crucian carp *Carassius carassius* or predatory sticklebacks *Gasterosteus aculeatus*. Experiments over three field seasons showed that great crested newt larvae spent a significantly higher proportion of time in vegetated compared to open water microhabitats when faced with caged predatory sticklebacks. Larvae exhibited no anti-predator response to crucian carp. Larvae showed no alterations in the degree of movement when in the presence of either fish species compared to controls. These results suggest that great crested newt larvae are able to distinguish predatory from benign fish species and exhibit behavioural plasticity to reduce predation risk.

8.2. Introduction

Fish often have detrimental effects on amphibian populations by direct predation on aquatic larvae (Brönmark & Edenhamn, 1994; Lecis & Norris, 2003; Orizaola & Braña, 2003). These effects have been documented in a wide range of locations and species (Beebee, 1996; Gamradt & Kats, 1996; Kats & Ferrer, 2003; Laurila & Aho, 1997; Saenz et al., 2003). Many larval amphibians exhibit a range of behavioural (Altwegg, 2002; Azevedo-Ramos et al., 1992; Relyea, 2004) and morphological (Relyea & Werner, 2000; Van Buskirk & McCollum, 2000) responses that reduce the effects of predation such as decreased activity and increased hiding in protected microhabitats. In experimental situations when in the presence of caged predators, many amphibian larvae exhibit behavioural adaptations such as a reduction in movement (Anholt et al., 2000; Laurila & Kujasalo, 1999) or altering of microhabitat preference (Mathis et al., 2008) which is thought to be induced by detection of predator chemical cues. When developing over a period of several weeks in the presence of caged predators, larvae exhibit morphological adaptations such as increased tail depth (Relyea, 2002b) or tail muscle depth and width (Teplitsky et al., 2003; Van Buskirk et al., 1997), both of which increase swimming speeds. The majority of these studies have involved anuran larvae (e.g. Smith et al., 2009; Stav et al., 2007; Van Buskirk, 2002) and relatively few have examined the potential for phenotypic responses in salamander or newt larvae.

Van Buskirk & Schmidt (2000) carried out experiments on palmate *Lissotriton helveticus* and alpine *Mesotriton alpestris* newts which showed that larvae altered their habitat use when in the presence of predatory dragonfly nymphs.

Individual larvae occupied more vegetated microhabitats when in the presence of predators which suggests that these species have the capacity to exhibit an anti-predator response. Studies on *Triturus* newts (e.g. *T. cristatus*) have revealed that this genus may be more vulnerable to fish predation than anurans (Orizaola & Braña, 2006). This is because the free-swimming larvae have no toxicity and often develop in freshwater habitats which contain fish.

Great crested newt larvae are particularly vulnerable to predation by fish (Beebee, 1996, 1997), notably sticklebacks (Beebee, 2007; English Nature, 2001a) due to their nektonic behaviour which leaves them vulnerable to attack. Large populations have been reported to decline to extinction when predatory fish have been added to great crested newt breeding ponds (Cooke, 1994). Research has shown that adult great crested newts may avoid laying eggs in ponds where fish are present (Beebee, 2007) and it appears that females use chemical cues to detect the presence of predatory fish (Malmgren, 2003). However, females are highly faithful to favoured breeding ponds (Beebee, 1996) and if fish are introduced to a pond, individuals may still continue to lay eggs in these sites (Beebee, 2007). Great crested newts, like all European newts, wrap their eggs in aquatic vegetation (Miaud, 1995), which may serve to reduce predation since eggs are concealed and less visible to attack. Eggs are also spread among vegetation more widely, which will also serve to reduce predation by the dilution effect. In addition, wrapping eggs reduces the damaging effects of UV-radiation (Marco et al., 2001). Therefore it appears that embryos have a degree of protection from predators, but no study has specifically examined whether great crested newt larvae exhibit anti-predator

responses when in the presence of predatory fish and whether they alter their behaviour depending on the species present.

This study looked at the behaviour of great crested newt larvae in a laboratory setting to see whether they: i) altered their microhabitat usage by spending more time in vegetation and ii) decreased levels of movement when in the presence of either caged predatory or non-predatory fish. Both these behaviours would be expected to be exhibited when larvae are in the presence of predators as they would serve to make larvae less vulnerable to predation. I hypothesised that great crested newt larvae would exhibit less or no anti-predator responses when in the presence of non-predators compared to predatory fish species.

Two species of fish were used in experiments: crucian carp *Carassius carassius* and three-spined stickleback *Gasterosteus aculeatus*. Crucian carp are related to goldfish *Carassius auratus*, have a dull brown colouration and grow to between 10 and 15 cm (Muus & Dahlstrøm, 1971). They typically feed on benthic animals and plant detritus (Tonn et al., 2004) and are thus considered benign to great crested newts (Kloskowski, 2009; Oldham et al., 2000). Sticklebacks are small carnivorous fish growing to between 5 and 8 cm long with three small dorsal spines. They are active predators, typically feeding on small animals such as invertebrate larvae, but they will also attack larger prey such as tadpoles (Muus & Dahlstrøm, 1971). All sticklebacks used in experiments were sub-adults to reduce any sex-linked effects on larval newt behaviour. This species is considered to have serious negative impacts on

great crested newt populations through direct predation on larvae (Oldham et al., 2000).

8.3. Methods

8.3.1. Collection of great crested newt larvae

Great crested newt larvae were collected from a local pond using standardised dip-netting procedure (Griffiths & Langton, 2003). Captured larvae were placed into a tank of pond water on a laboratory bench at room temperature (20 °C ± 3 °C), subject to natural light:dark cycles for 24 hours prior to experiments taking place. Larvae were fed with zooplankton sourced from a local pond.

8.3.2. Acquisition and description of fish species

Crucian carp were captured in 2007 from the same breeding pond as great crested newt larvae with standard dip netting procedure and placed in a tank of pond water. Three-spined sticklebacks were obtained in 2009 from a commercial supplier (Blades Biological Supplies) two weeks prior to experiments taking place and placed in a tank of aged tap water. Both tanks were placed on a laboratory bench at room temperature (20 °C ± 3 °C) and were subject to natural light:dark cycles. Fish were fed daily with commercial fish flakes (TetraFin®) which contains mainly fish derivatives, cereals, algae, molluscs and crustaceans.

8.3.3. Experiment 1 procedure

This experiment was designed to test the effects of caged crucian carp on the microhabitat selection choice of great crested newt larvae. Ten opaque plastic containers (55 cm L x 45 cm W x 33 cm D) were filled with aged tap water

(maximum depth 28 cm) and placed on a laboratory bench at room temperature (20 °C ± 3 °C) and were subject to natural light:dark cycles. Containers were divided visually into four equal quadrants. Within two opposite quadrants were placed artificial egg strips to mimic vegetation (Figure 8.1). Real vegetation was not used to avoid confounding variables such as the addition of other organisms (e.g. algae) and oxygenation, which may affect larval behaviour. The artificial vegetation floated throughout the water column. Larvae therefore had two microhabitat choices: open water and 'vegetation'. A transparent, cylindrical plastic container (30 cm L x 9 cm C) with perforations (0.1 mm diameter) was placed in the centre of each tank to house the fish. The perforations would allow any fish chemical cues to exit the container but not allow predation of fish on great crested newt larvae.

Figure 8.1. Photograph of one experimental tank showing artificial vegetation and open water microhabitats.

One hour prior to experiments, five sticklebacks were captured from the holding tank and one placed in each of five randomly assigned tanks, within the plastic containers, and left to acclimatise. The remaining five tanks were left without fish to act as controls. Ten great crested newt larvae were randomly taken from

the holding tank and their total length was measured to the nearest 1 mm using a plastic ruler. The stage of development of each larva was estimated using a staging table in Gallien & Bidaud (1959). The estimated stage range of individuals was 42 to 44 in this procedure.

One larva was placed into the centre of each experimental tank one hour after the fish were introduced and left to acclimatise for 15 minutes. The microhabitat choice of each larva (open water or vegetation), as well as the position in water column (pelagic or benthic), was noted every 15 minutes for the next three hours.

At the end of each trial the sticklebacks were removed and returned to their holding tank. Larvae were returned to their pond of capture. All tanks were emptied and cleaned before refilling for a second trial, which was performed on a different date. A total of three trials were carried out.

8.3.4. Experiment 2 procedure

The procedure was exactly the same as experiment one, except that sticklebacks were used instead of crucian carp. In addition, due to the experiments being carried out later in the season, only larger larvae, stage 50 to 53 could be obtained.

8.3.5. Experiment 3 procedure

These experiments were designed to test the degree of movement in great crested newts when in the presence of caged sticklebacks. Ten experimental tanks were set up as in experiment 1. No artificial vegetation was added but

instead tanks were visually divided into 6 transverse sections using string (Figure 8.2). Each division was labelled between 1 and 6. One transparent plastic container with perforations was placed into each tank at one end. A metal spoon was added to prevent the bottle rolling along the bottom of the tank. This was not thought to affect the behaviour of either fish or great crested newt larvae.

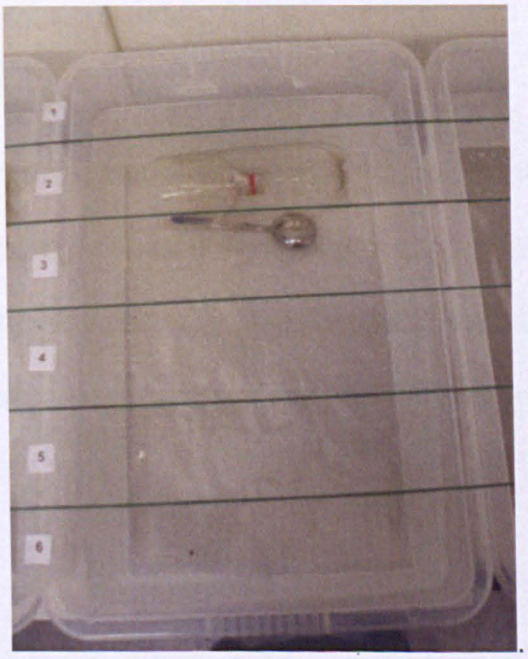


Figure 8.2. One of the tanks in experiment 3 showing the perforated plastic bottles and six visually marked divisions across each tank.

One hour prior to the start of the trials five sticklebacks were taken from the holding tank and one placed in each of five randomly assigned tanks within one of the perforated containers. The remaining five tanks were left empty of fish to act as controls but still had the containers and spoons. As in experiment 1, ten great crested newt larvae were taken from their holding tank, measured and stage determined. Each was placed into the centre of each container and allowed to acclimatise for 15 minutes. The division of each tank that each larva was present in was noted every 15 minutes for three hours.

At the end of the experiments sticklebacks were removed and returned to holding tanks. Larvae were returned to their pond of capture. All tanks were emptied and cleaned before refilling for a second trial, which was performed on a different date.

8.3.6. Data analysis

Experiments 1 & 2

The percentage of occasions that each larva was present in either open water versus vegetation or pelagic versus benthic was calculated. A two-way ANOVA was applied to arcsine-square root transformed proportion data to test for a difference in the mean percentage of times larvae were present in each microhabitat.

Experiment 3

The percentage of occasions that each larva was observed to move, along with the total number of divisions moved by each larva made was calculated. A two-way ANOVA was applied to arcsine-square root transformed proportion data to test for a difference in the mean percentage of times larvae were present in each division of the tank and degree of movement of larvae.

8.4. Results

8.4.1. Experiment 1. Microhabitat preferences of larvae when in the presence of caged crucian carp

Larval size varied between 25 and 32 mm; stage of development was between 42 and 44. Larvae spent a significantly larger proportion of their time in artificial vegetation compared to open water microhabitats in both fish and control treatments (Figure 8.3) ($F_{1,47} = 17.62$, $p < 0.001$). Larvae spent between 24 and 28% of occasions in open water compared to between 67 and 69% of the time in vegetated microhabitats.

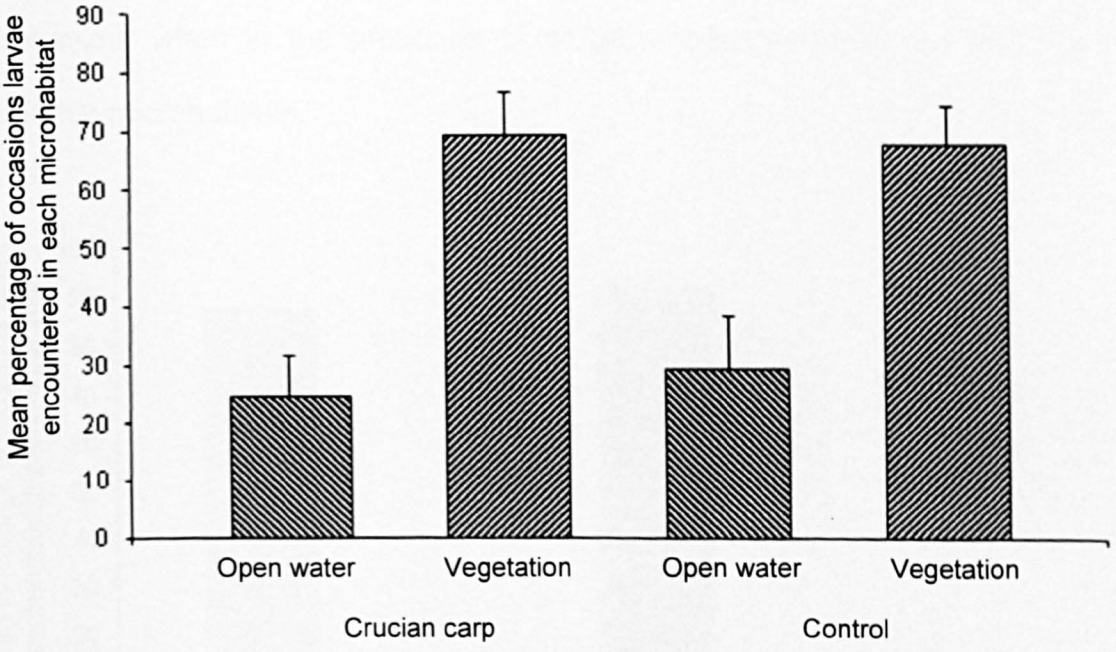


Figure 8.3. Mean percentage of occasions larvae were encountered in open water or vegetation microhabitats in crucian carp and control treatments.

There was no significant difference in the behaviour of great crested newt larvae when in the presence of crucian carp ($F_{1,47} = 0.29$, $p = 0.87$) compared to controls. There was also no interaction between fish and vegetation ($F_{1,47} = 0.23$, $p = 0.63$). This indicates that larvae did not respond to the presence of crucian carp and exhibited no obvious anti-predator behaviour.

Larvae spent a significantly larger proportion of time in pelagic compared to benthic microhabitats in both treatments (Figure 8.4) ($F_{1,47}$ = 263.91, $p < 0.0001$). Larvae occupied benthic microhabitats on only between 6 - 7% of occasions compared to 87 - 93% of the time as pelagic, in both treatments. There was no significant difference in the behaviour of great crested newt larvae when in the presence of crucian carp ($F_{1,47}$ = 2.11, p = 0.15) nor interaction between fish and vegetation. There was also no interaction between fish and vegetation ($F_{1,47}$ = 0.47, p = 0.50). Therefore larvae did not alter their behaviour when in the presence of crucian carp by spending more time in benthic microhabitats.

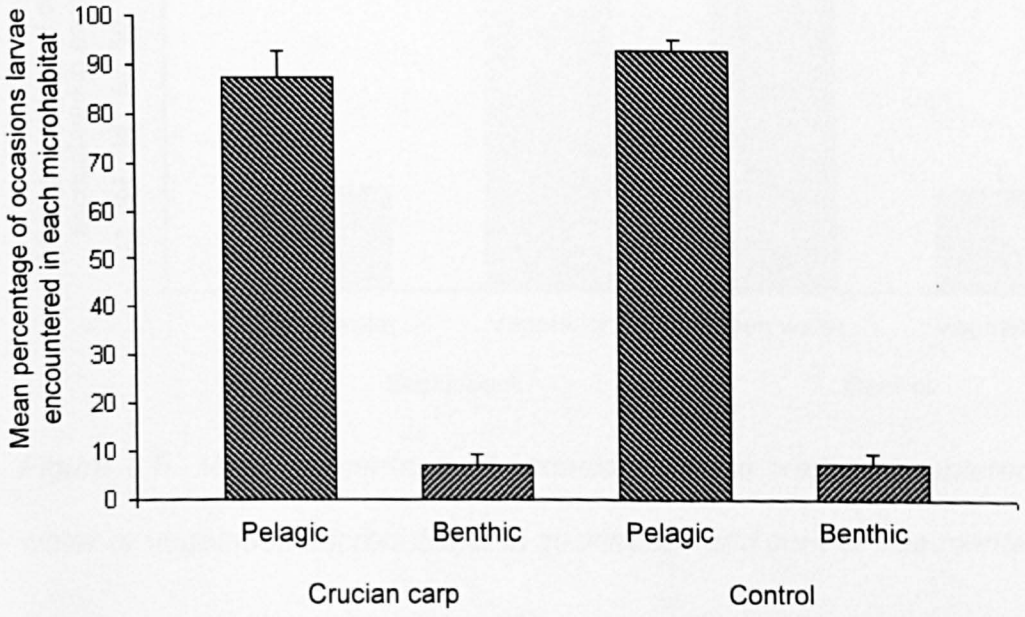


Figure 8.4. Mean percentage of occasions larvae were encountered in pelagic or benthic microhabitats in crucian carp and control treatments.

8.4.2. Experiment 2. Microhabitat preferences of larvae when in the presence of caged sticklebacks

Total larval length varied 42 to 49 mm and the stage of development was between 50 and 53. This is older than the larvae in experiment 1, which were at stage 42 to 44. When in the presence of sticklebacks great crested newt larvae spent a significantly larger proportion of their time in vegetated microhabitats (Figure 8.5) (fish and vegetation interaction: $F_{1,39} = 62.36$, $p < 0.0001$). These results suggest that great crested newt larvae can detect predatory sticklebacks and respond by utilising more vegetated microhabitats.

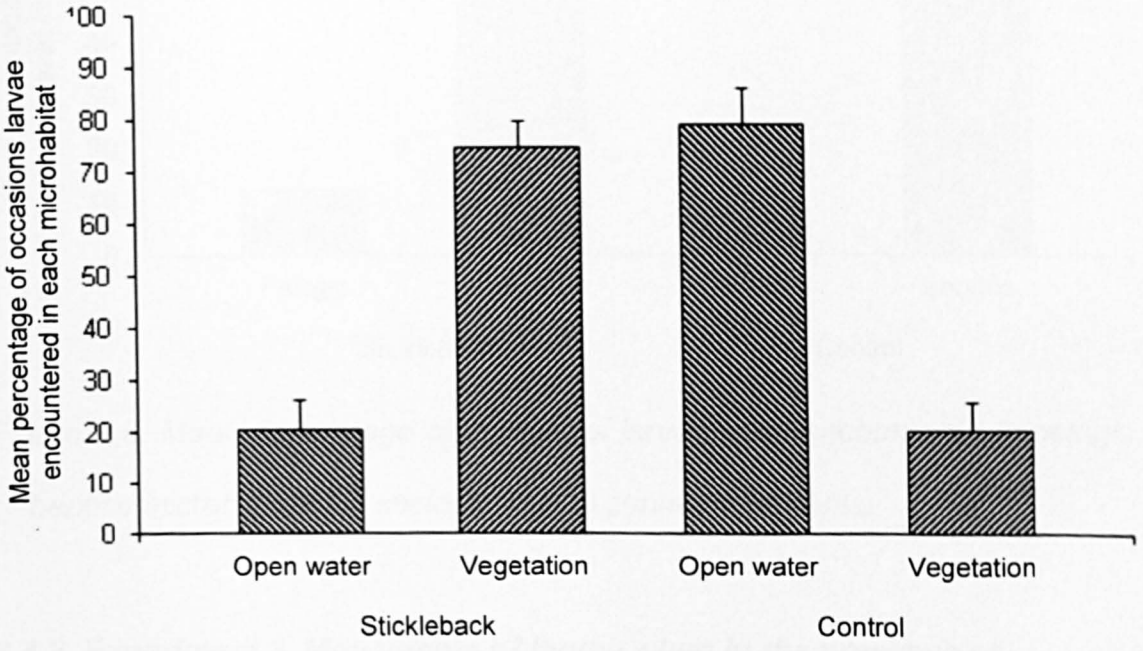


Figure 8.5. Mean percentage of occasions larvae were encountered in open water or vegetation microhabitats in stickleback and control treatments.

Great crested newts were significantly more likely to spend their time in benthic compared to vegetated microhabitats across all treatments (Figure 8.6) ($F_{1,39} = 177.37$, $p < 0.0001$). When in the presence of sticklebacks, great crested newt larvae spent between 8 and 12% of occasions in pelagic and between 87 and 90% of their time in benthic microhabitats. Larvae did not alter their behaviour when in the presence of sticklebacks and spent equally large proportions of

their time in benthic microhabitat compared to controls ($F_{1,39}$ = < 0.01, p = 0.99). These results suggest that there is no effect of the presence of stickleback on whether larvae were either benthic or pelagic.

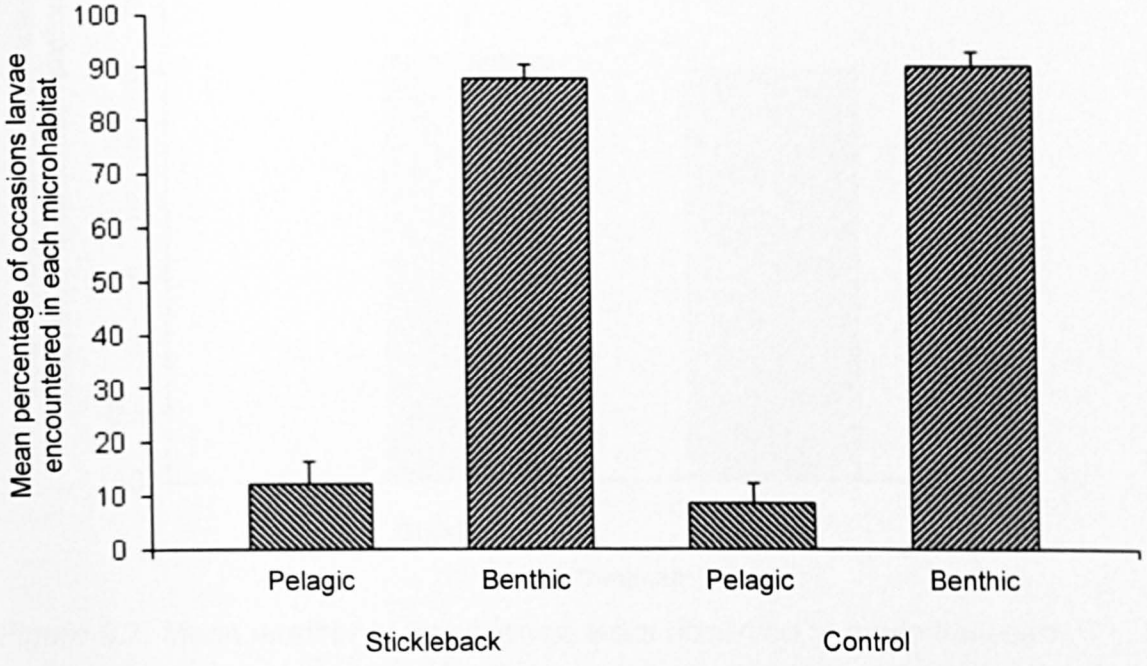


Figure 8.6. Mean percentage of occasions larvae were encountered in pelagic or benthic microhabitats in stickleback and control treatments.

8.4.3. Experiment 3. Movements of larvae when in the presence of predatory sticklebacks

There was no significant difference in the number of times larvae were observed to move between sections (Figure 8.7) ($F_{1,47}$ = 0.042, p = 0.84). Larvae were only observed to make, on average, three movements between divisions in each trial in both control and stickleback treatments. This indicates there was no increase or decrease in the number of movements made by larvae in either treatment. There was also no significant difference in the total number of sections moved in either control or stickleback treatments (Figure 8.8). These

data give an indication of the distance moved by larvae. On average, larvae moved between 7 and 8 sections over the course of each trial.

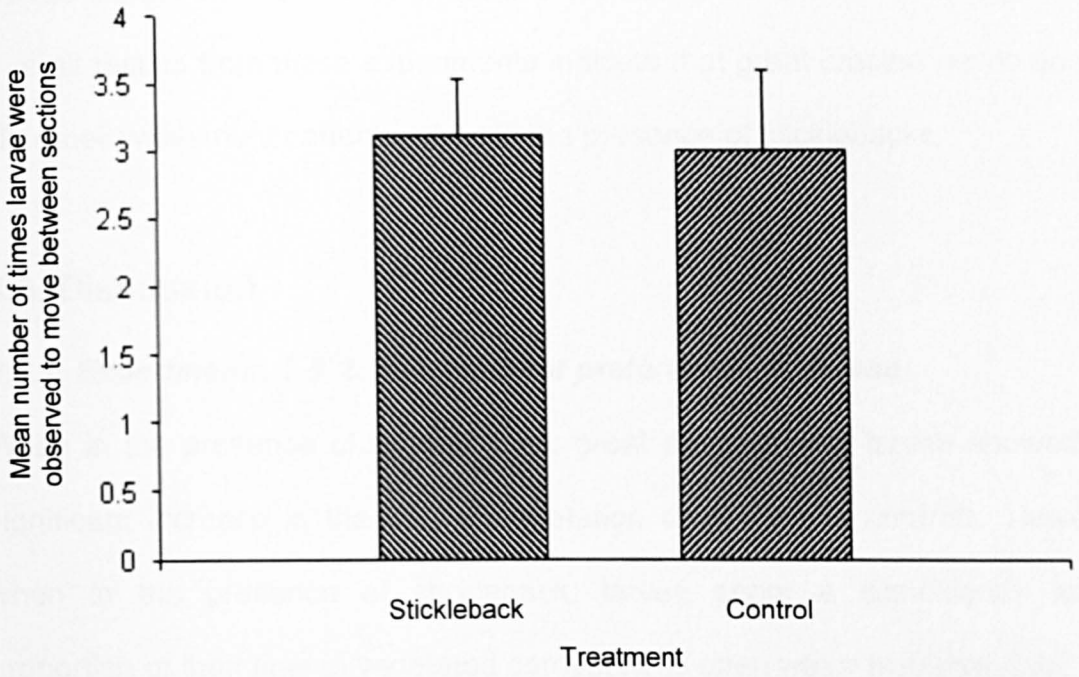


Figure 8.7. Mean number of times larvae were observed to move between sections in stickleback and control treatments.

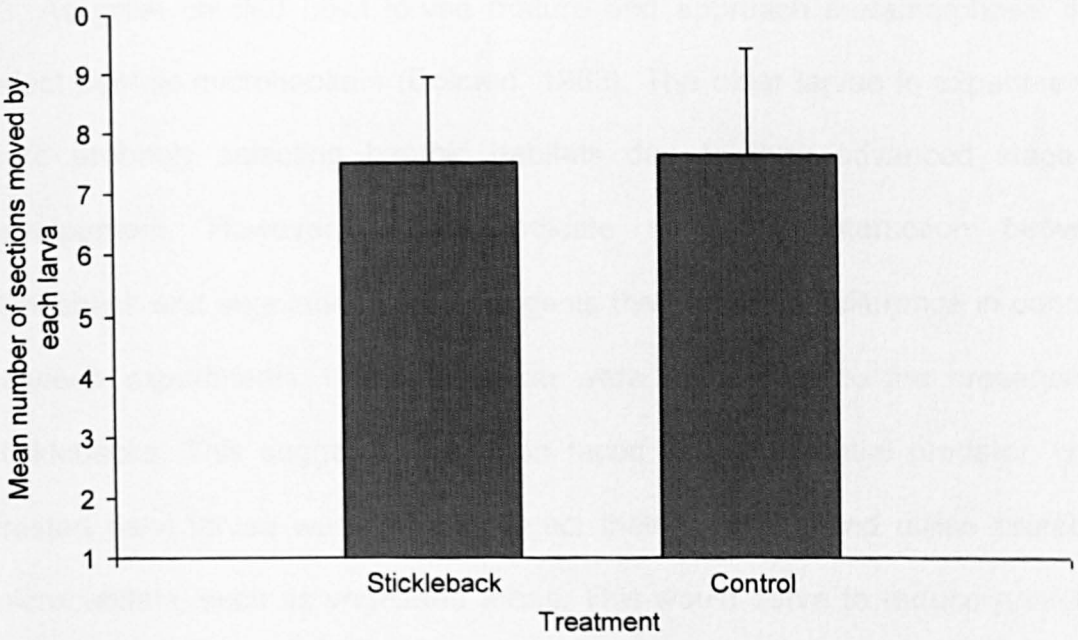


Figure 8.8. Mean number of sections moved by great crested newt larvae in stickleback and control treatments.

Larvae did not show any differences in the distance travelled in either treatment. Overall results from these experiments indicate that great crested newts do not alter their movement patterns when in the presence of sticklebacks.

8.5. Discussion

8.5.1. Experiments 1 & 2: Microhabitat preferences of larvae

When in the presence of crucian carp, great crested newt larvae showed no significant increase in the use of vegetation compared to controls. However when in the presence of stickleback, larvae spent a significantly larger proportion of their time in vegetated compared to open water microhabitats. The behaviour of control larvae also changed between the two experiments. This may be because the larvae in experiment 1 were between stages 42 and 44 (Gallien & Bidaud, 1959), whereas larvae in experiment 2 were at stages 52 to 53. As great crested newt larvae mature and approach metamorphosis they select benthic microhabitats (Dolmen, 1983). The older larvae in experiment 2 were probably selecting benthic habitats due to their advanced stage of development. However, results indicate a strong interaction between stickleback and vegetation which suggests that despite a difference in controls between experiments 1 and 2, larvae were responding to the presence of sticklebacks. This suggests that when faced with a potential predator, great crested newt larvae were able to detect their presence and utilise protected microhabitats, such as vegetated areas. This would serve to reduce predation risk when predators such as sticklebacks are present, as has been recorded in other *Triturus* newts (Van Buskirk & Schmidt, 2000). Results also indicate that

great crested newt larvae appear to be able to distinguish between fish which are benign (crucian carp) and those that are predatory (sticklebacks). Relyea (2001) states that prey may not develop responses if the threat of the predator is too low. It appears that the tadpoles were determining the relative risk of the predators and altering their behaviour when at the highest threat. Therefore great crested newt larvae may be determining the relative risks of the fish species present and assessing the threat posed by crucian carp as being too low.

Larvae in experiment 1 spent a significantly larger proportion of their time in pelagic compared to benthic microhabitats, whereas larvae in experiment 2 utilised benthic habitats more frequently. There was no impact of predator type on whether larvae were benthic or pelagic. The differences observed may therefore be linked to the age of the great crested newts. The older larvae in experiment 2 were probably selecting benthic habitats due to their advanced stage of development.

The cues used to detect potential fish predators may be visual or chemical. Larval amphibians have poor eyesight when underwater (Griffiths, 1996) so it is unlikely that larvae were utilising visual recognition. However fish are known to produce chemicals or kairomones from their skin which are unique to different species (Weber, 2003) making it possible that larvae can detect fish using chemical recognition. Barks et al. (2000) demonstrated that fish produce chemical cues which elicited anti-predator responses in *Daphnia* species. Experiments on anurans suggest that species use different chemical cues in detecting predators. Kiesecker & Blaustein (1997) suggest that naïve prey may

not exhibit anti-predator behaviour when exposed to a new predator, but will respond to a native one. Both sticklebacks and crucian carp are native to the British Isles and newts will have coevolved with these fish species. Therefore great crested newts may be using an evolved ability to detect unique chemicals from sticklebacks which are different from those produced by crucian carp. This may enable them to exhibit anti-predator behaviour.

In addition to detecting predators directly, amphibians may also use cues from conspecifics. Van Buskirk & Arioli (2002) found that pool frog *Rana lessonae* tadpoles would elicit a greater response if they were exposed to the chemicals of crushed conspecifics. This is due to an alarm signal released when the individual is killed (Jordão & Volpato, 2000). Ethically this was not possible in my experiments but great crested larvae may have shown an enhanced response if other larvae had been initially killed by predators.

8.5.2. Experiment 3: Movements of larvae

There was no significant difference in the mean number of observed movements made between divisions by larvae in either control or in the presence of sticklebacks. On average all larvae were only observed to make three movements over the course of each three-hour trial. Results also show that there was no significant difference in the mean number of sections moved by larvae. On average, when movements were made, larvae only moved a total of 7 sections. Firstly, these results show that great crested newt larvae were very sedentary and, secondly, that they made no alterations in the number or degree of movements when in the presence of sticklebacks. Previous studies have shown that many prey species reduce their movements when in the

presence of fish (Relyea, 2004; Relyea & Mills, 2001; Sih et al., 1992) as this makes individuals less obvious (Azevedo-Ramos et al., 1992). The majority of these studies were conducted on anuran larvae which are predominantly detritus or herbivorous feeders, grazing continuously on pond substrates (Harrison, 1987). Any reduction in movement would be noticeable due to this near-continuous movement. Larval newts are carnivorous, adopt a sit-and-wait feeding strategy and spend a large proportion of their time motionless. Any further reduction in movement would therefore be less noticeable and would not significantly reduce predation risk. Therefore, although great crested newt larvae spent significantly more time in vegetation compared to open water (Experiment 2), they do not appear to make alterations to movement, probably because they already spend the majority of time motionless. Individuals would gain no extra survival advantage by making any further reductions in movement.

8.5.3. Critique of methods

These experiments had a number of limitations:

i) Different aged larvae were used in experiments 1 & 2. The difference in age was enough to alter the preferred microhabitat usage of larvae from pelagic to benthic. There may also be a difference in how and to the extent larvae of different ages respond to predators. Further research is required into this area to see whether younger life stages exhibit a greater or lesser response to stickleback cues.

ii) Experiments only used one predatory fish in each container. A greater number of fish may have given a larger response (Relyea, 2004), which would

make drawing conclusions more effective. Future experiments may look at altering the degree of perceived predation risk by increasing the number of sticklebacks used.

iii) No crucian carp were available for Experiment 3 due to difficulties in capturing enough adults in breeding ponds.

iv) These experiments were all laboratory-based and may not reflect behaviour in the natural environment. Further experiments in the natural environment would be useful to determine how larvae behave in ponds.

8.6. Conclusions

i) Great crested newt larvae appeared to be able to differentiate between the chemical cues from a predatory and non-predatory fish. Larvae utilised vegetated microhabitats for a significantly greater proportion of the time when in the presence of predatory sticklebacks but not when faced with non-predatory crucian carp.

ii) Larvae spent more time pelagic in younger stages of development and become benthic in later stages, which confirm the observations by Dolmen (1983).

iii) Larvae do not make any changes to the number or degree of movements when in the presence of predatory sticklebacks. This may reflect their sit-and-wait feeding strategy so reducing movements would have little survival benefit.

Chapter 9. The effects of intra-specific competition on oviposition behaviour in female great crested newts *Triturus cristatus*.

9.1. Abstract

Oviposition site selection potentially has important fitness implications for the survival of embryos and larvae. Three experiments over two years examined the egg-laying choices of female great crested newts *Triturus cristatus*. Females were given the choice of laying eggs on artificial egg strips that were either empty (clean) or occupied with conspecific eggs. Results showed that female great crested newts preferred to lay eggs on clean compared to occupied strips. This may be due to increased effectiveness in wrapping eggs and decreased risks of fungal infection transfer from existing eggs. However there was no significant difference in the number of eggs laid on clean compared to occupied strips. This indicates that there may be a trade-off between laying eggs on clean compared to occupied strips. There was no significant correlation between the number of eggs laid on strips and the number already present, suggesting that females do not just lay on strips until they are full. Results add to the evidence that great crested newts exhibit a non-random distribution in egg laying and demonstrate an ability to make choices about oviposition site selection.

9.2. Introduction

Since the quality of oviposition sites varies, the choice of oviposition site may affect fitness (Mokany & Shine, 2003) and result in competition within and among amphibian species for access to high quality sites (Spence et al., 2007). Many species of amphibian lay hundreds or thousands of eggs per breeding attempt (Duellman & Trueb, 1986) but survival is estimated to be low. For example, in European newt species only 0.03 to 0.16% of eggs survive (Bell & Lawton, 1975; Miaud, 1993). Few of these species exhibit parental care (Sullivan et al., 1995) so there is strong selection pressure on females to select optimal oviposition sites (Spence et al., 2007. The advantages associated with the selection of suitable oviposition sites include decreased risk of mortality due to cannibalism, predation, competition or desiccation (Goldberg et al., 2006). Several amphibian species, including the Californian newt *Taricha torosa* (Kaplan & Sherman, 1980) and the smooth newt *Lissotriton* (*Triturus*) *vulgaris* (Tóth et al., 2011) have been observed to consume the eggs of conspecifics. Predation of great crested newt eggs by fish (Beebee, 1996; English Nature, 2001) and smooth newt eggs by caddisfly larvae (Bell & Lawton, 1975) has also been noted, which is likely to decrease reproductive success. Water levels will change throughout the breeding season resulting in the potential for eggs to be left stranded, especially in species which lay in ephemeral water bodies, such as the natterjack toad *Epidalia* (*Bufo*) *calamita* (Stevens & Baguette, 2008). Therefore amphibians are expected to lay their eggs in sites that are optimal for development and subsequent larval survival (Resetarits, 1996).

Studies on amphibian species have shown that individuals attempt to lay eggs in sites that optimise survival but strategies vary with species (Marsh & Borrell, 2001). For example, female Cope's tree frog *Hyla chrysoscelis* discriminate

among breeding sites with females laying in ponds with the fewest predators (Resetarits, 1996). Wood frogs *Rana sylvatica* lay eggs in warmer areas of ponds which results in faster growth and also place eggs in close contact to enhance thermal insulation (Seale, 1982). Túngara frogs *Physalaemus pustulosus* choose to lay their eggs in puddles which have no eggs already present to reduce competition as they desiccate rapidly (Dillon & Fiaño, 2000). Therefore the optimum strategy for each species differs depending on the particular combination of biotic and abiotic factors at a given location.

Triturus newts lay between 100 and 300 eggs per female across each breeding season (Langton et al., 2001) which is relatively few compared to anurans. Females may lay several eggs per piece of vegetation wrapping each egg individually in a leaf, resulting in a concertina effect to the leaf (Langton et al., 2001). Egg wrapping reduces the risks of predation (Miaud, 1994; Orizaola & Braña, 2003) and damage by ultraviolet radiation (Marco et al., 2001). Once the eggs are wrapped, they are left by females to develop without parental care. Not all eggs will hatch as approximately 50% of great crested newt embryos will abort at the tail bud stage due to non-disjunction of chromosome 1 (Horner & MacGregor, 1985), which may place extra pressure on females to select suitable oviposition sites. In addition, newts may consume the eggs of conspecifics. *Lissotriton vulgaris* has been observed to exhibit conspecific oophagy before laying eggs (Tóth et al., 2011). In some situations individuals appear to be able to distinguish kin from non-kin (Tóth et al., 2011). This behaviour is thought to reduce competition for egg-laying sites and also provide the female with extra nutrition to produce her own eggs.

Little research has examined how and to what extent *Triturus* newts select suitable sites to lay eggs in relation to intra-specific competition. Most studies have examined the plant species and substrate support preferences. For example Miaud (1995) recorded *Triturus* newts selecting specific plant species and locations in ponds to enhance embryo survival. Norris & Hosie (2005) observed *Lissotriton vulgaris* and *L. helveticus* make sniffing movements before laying eggs, suggesting that females select for specific cues and locations to lay their eggs. The marbled pygmy newt *Triturus pygmaeus* has been observed to avoid laying eggs on substrate which already has eggs attached due to the limited substrate available (Jehle et al., 2000). This also implies that some selection of egg-laying sites takes place, but none of these studies have looked specifically at the effects of the presence of conspecific eggs on oviposition site selection. In light of the lack of research in this area, especially in great crested newts, I investigated the sites used by females when faced with a choice between occupied or clean egg strips.

9.3. Methods

9.3.1. Collection and housing of female great crested newts

Adult female great crested newts were captured in funnel traps at a local pond (see Chapter 2 for details of capture) and transferred to a tank of pond water (55 cm L x 45 cm W x 33 cm D) in a laboratory subject to natural light:dark cycles at 20 °C for 24 hours prior to experiments. Adults were fed zooplankton whilst in the holding tank but were not fed during the experimental procedure. Females were returned to their local pond of capture immediately after taking part in experiments.

9.3.2. Experimental procedure

Ten opaque plastic containers (55 cm L x 45 cm W x 33 cm D) were filled with aged tap water and placed on a laboratory bench in the same conditions as the holding tank. Artificial egg-laying substrate consisting of black plastic bags cut into 1.5 cm wide x 30 cm length strips were placed into a local great crested newt breeding pond. This species is known to readily lay on artificial substrate (Miaud, 1995). Strips were placed in the pond in mid-April during the peak egg-laying season in this area (personal observation). Half the strips were left available for great crested newt females to lay eggs on while the remaining strips were placed into a mesh bag (50 x 50 cm; mesh size < 0.5 mm), preventing oviposition. After 24 hours all the strips were removed from the pond; fifty strips containing eggs (occupied strips) were randomly assigned to each of the 10 experimental tanks, five per tank. The number of eggs on each strip was counted by the presence of a fold and recorded as the starting number of eggs on each occupied strip. Eggs were left wrapped to reduce the impact of ultraviolet radiation and to mimic natural conditions. A further five empty control strips were placed into each tank, alternating with occupied strips (Figure 9.1).

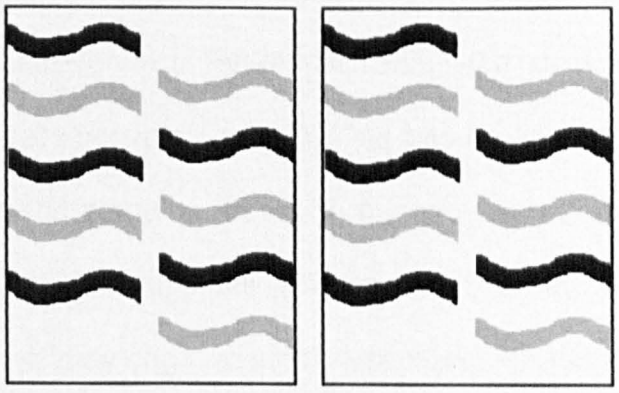


Figure 9.1. Diagram to show two tanks with artificial strips set-up for the oviposition experiments. Each tank contained 10 strips: five occupied (black) and five clean (grey).

After females had been in holding tanks for twenty-four hours, one great crested female was introduced to each tank at 17:00 h and lids placed over the top to prevent escape. Females were left overnight as this is the time they are most active and likely to lay eggs (Dolmen, 1983). A gap of 5 cm depth was left at the surface to allow enough air for breathing. Females were left until 09:00 h the following morning, and then returned to breeding ponds. The number of eggs on previously occupied and clean strips was counted. Any lost eggs on strips were noted. Egg strips were then returned to the local pond of capture to allow continuation of development. Experimental tanks were emptied, cleaned and refilled with new water to allow a repeat of the experiment. Different females and strips were used in each trial. A total of three trials were performed in total on 14th, 21st and 28th April, 2010.

9.3.3. Data analysis

The number of strips with new eggs was recorded for each tank and a two-way Analysis of Variance (ANOVA) was performed to determine whether females laid new eggs on clean strips more often than on occupied strips (i.e. do females prefer to lay eggs on clean strips compared to occupied strips?) and to see if there was a difference in female behaviour (i.e. do some females prefer clean strips and others occupied ones?). This was determined by examining the difference between the samples (tanks) in the two-way ANOVA. If samples are significantly different, this suggests differences in female behaviour between tanks. The number of new eggs on strips was then counted for each tank and a one-way ANOVA performed to determine whether there was a significant difference in the number of new eggs laid on clean versus occupied strips (i.e. do females lay a greater number of eggs on clean compared to occupied

strips?). Finally, a linear regression was performed to determine whether there was a significant relationship between the number of eggs on strips at the beginning and end of experiments (i.e. do females lay fewer eggs on strips which already have a high number of eggs attached?).

9.4. Results

9.4.1. Presence of eggs on clean and occupied egg strips

A significantly higher number of clean strips contained new eggs compared to occupied strips after experimentation ($F_{1,39}$ = 5.52, p = 0.02). On average, females laid eggs on 3 ± 0.35 clean strips per tank compared to 2.1 ± 0.35 occupied strips. There was no significant variation in the behaviour of individual females ($F_{19,39}$ = 1.50, p = 0.19). These results suggest that females prefer to lay eggs on clean rather than occupied strips. However, females do not appear to completely avoid strips with eggs already present, indicating that there may be benefits of laying eggs on occupied strips.

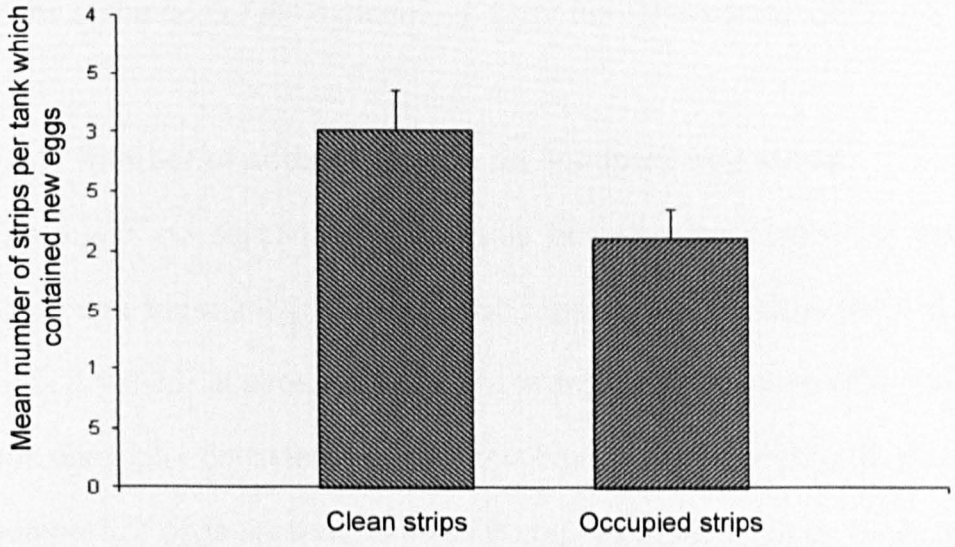


Figure 9.2. The mean number of strips per tank which contained new eggs at the end of the experiment.

9.4.2. Number of additional eggs on clean and occupied egg strips

There was no significant difference in the number of new eggs laid on clean compared to occupied strips ($F_{1,199}$ = 1.84, p = 0.18). Females laid on average 1.77 ± 1.3 eggs on clean strips compared to 1.31 ± 0.25 on occupied strips. This demonstrates that, although preferring to lay eggs on clean strips (9.4.1.), females do not necessarily lay a greater number of eggs on clean strips.

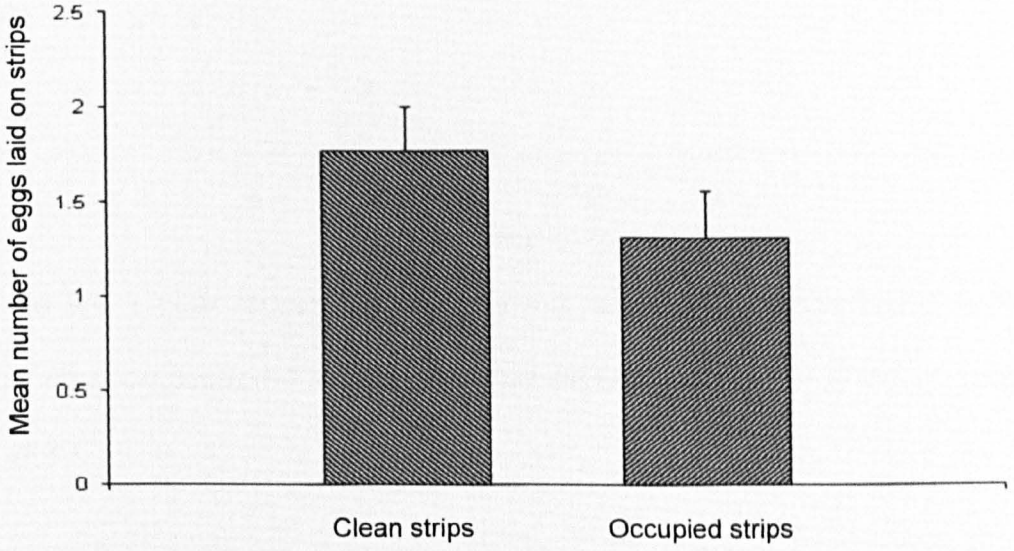


Figure 9.3. The mean number of eggs laid on clean compared to occupied strips at the end of the experiment. Error bars denote standard error

5.4.3. Number of additional eggs on occupied egg strips

There was no significant relationship between the number of initial eggs on strips and the number of additional eggs laid by females (R^2 = 0.037, $F_{1,38}$ = 1.45, p = 0.23). It appears that when laying eggs on occupied strips, females do not take into consideration the number already present. If there are large numbers of eggs present, females do not lay fewer eggs on these strips (Figure 9.4).

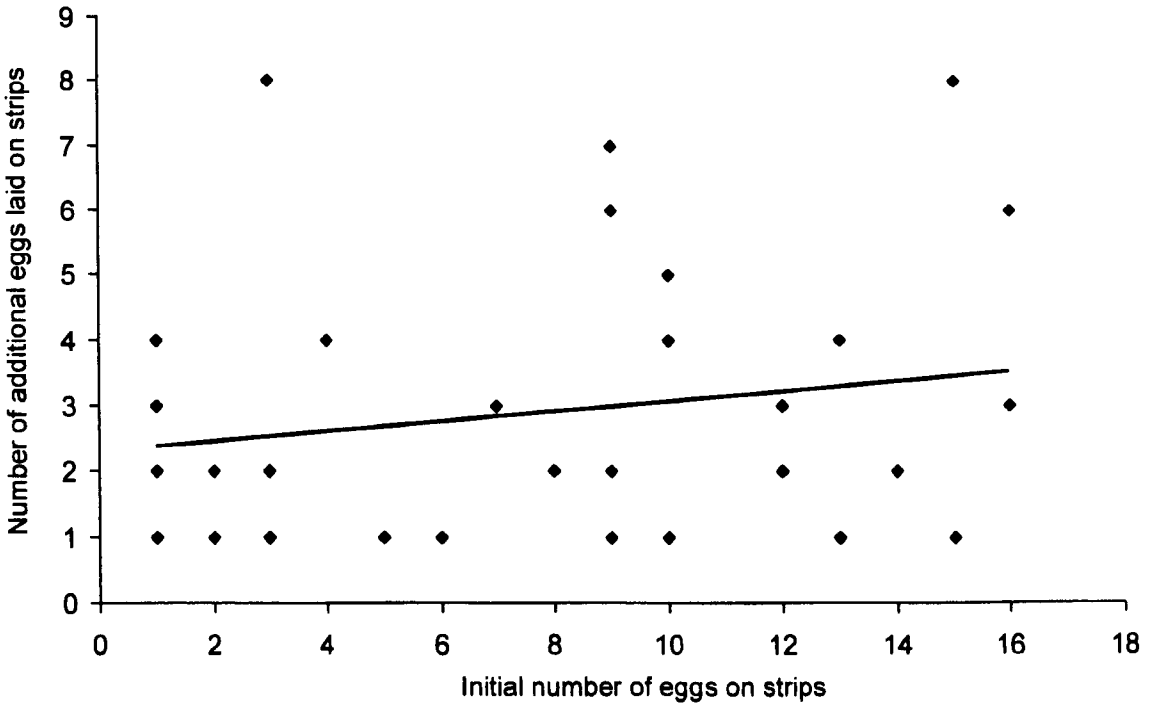


Figure 9.4. Linear regression to show the relationship between the number of initial eggs on occupied strips compared with the number of additional eggs laid on strips.

9.5. Discussion

9.5.1. Presence of eggs on clean and occupied egg strips

Results from these experiments indicate that female great crested newts are more likely to lay eggs on clean compared to occupied strips. However females do not completely avoid laying eggs on strips with those of conspecifics already attached, suggesting there may be some benefit of laying on occupied strips. The benefits of ovipositing on an unoccupied substrate are: reduced competition for oxygen in developing embryos, greater adhesion and effectiveness of egg wrapping, and decreased risk of transfer of fungal pathogens from conspecific eggs (Green, 1999). Like all eggs, those of great crested newts need sufficient oxygen for development and low oxygen levels can kill or retard amphibian embryo development (Bradford, 1993; Olivier &

Moon, 2009; Slos & Stoks, 2008; Warkentin, 2001). Therefore there may be a selective advantage for laying eggs in water with higher oxygen levels. One way to achieve this is to lay eggs in shallow water where there is greater oxygen diffusion from the atmosphere (Miaud, 1995). Alternatively, laying eggs on clean substrate, away from the presence of conspecific eggs may also place these embryos in higher oxygen levels.

There is little previous research suggesting that eggs adhere more effectively to clean substrate. However newts have been observed to take great care over site selection and laying of eggs (Norris & Hosie, 2005). Miaud (1995) found that in natural habitats, great crested newts preferred to lay their eggs on one particular species of plant, *Nasturtium officinalis,* where 95% of eggs were located. In the same study, artificial experiments showed that females seemed selective in oviposition sites, only laying on thicker egg supports. In addition, Gustafson et al. (2007) found that great crested newts prefer certain plant species, in this case floating sweet grasses, *Glyceria* sp. and water mint. Therefore great crested newts favour certain substrates for egg-laying and this may depend on the effective adhesive, a large enough surface area to cover eggs, ease of folding and proximity to the surface. In common with other newts, great crested females wrap their eggs in vegetation which protect the embryos from predation, UV-B radiation and algal growth (Orizaola & Braña, 2003). Effective adhesion and folding of eggs may therefore benefit embryo survival. By selecting clean strips, females may enhance the survival of eggs by ensuring effective folding of strips.

Fungal infections are known to cause developmental problems in eggs (Blaustein et al., 1996; Johnson et al., 2002; Warkentin, 2001), particularly *Saprolegnia* species (Blaustein et al., 1994a; Daszak et al., 2003; Fernandez-Beneitez et al., 2008), which has led to high mortality in some species. When infected, *Saprolegnia* sp. fungal infection spreads in a wave-like fashion across egg masses killing up to 95% of the embryos (Blaustein et al., 1994a). This is probably due to the rupturing of the vitelline membrane and the subsequent smothering of the embryo by hyphae (Blaustein et al., 1994). Spread of the fungus can be achieved through growth of hyphae (Wood & Willloughby, 1986). Consequently, eggs in close proximity to others may have an increased chance of infection. Green (1999) noted that fungal infections spread rapidly between smooth newt eggs when they were in close proximity, resulting in high mortality. Growth of such fungi inhibits oxygen transfer resulting in developmental retardation, death (Post, 1988 in Jalilpoor et al., 2006) or possibly hatching (Warkentin, 2001). The latter may result in small, vulnerable larvae which are prone to predation. Therefore there are selective advantages for females to avoid or limit inter-fungal transfer on their embryos. One way of achieving this would be to avoid substrate where eggs are already present.

As demonstrated in this study, females do not completely avoid occupied strips which suggest that there may be some benefits associated with laying eggs close to those of conspecifics. One main benefit is safety in numbers due to the dilution effect, resulting in reduced predation (Iwai et al., 2007). A predator such as a caddis fly larva attacking eggs on a piece of vegetation leaf is less likely to consume eggs of a particular female if eggs from conspecifics are also attached. A second benefit of being in close proximity to conspecifics is that

when larvae hatch, being in the presence of many larvae may lead to increased survival due the dilution effect (Iwai et al., 2007). In anurans, this may increase the developmental rate of embryos (Duellman & Trueb, 1986). Although newts lay eggs singly and will not benefit from increased developmental rate, great crested newts may prefer to lay on occupied strips for the dilution effect in hatching larvae.

9.5.2. Number of additional eggs on clean and occupied egg strips

Results show that there was no significant difference in the number of additional eggs laid on clean compared to occupied strips. One reason for this may be that the perceived costs and benefits of laying on clean compared to occupied strips varies by female, resulting in some preferring to lay more eggs on clean strips and vice versa.

It was noted that no females exhibited conspecific oophagy in any of the experiments. This may have been because females were not hungry, that they don't view the other eggs as a threat, or a by-product of females being in captivity. It is possible that females do not regard conspecific eggs as a threat, especially if egg-laying sites are not in short supply. In addition females may regard the presence of conspecific eggs as an indication of suitable habitat. Further experiments examining the responses of females when oviposition sites are limiting would be an interesting avenue for future research.

9.5.3. Number of additional eggs on occupied egg strips

A negative correlation between the number of eggs already on occupied strips and the number laid during the experiment would be expected if females simply lay eggs on a strip until there is no more space and then move to the next one. No such correlation was detected. Females did not lay fewer eggs on crowded strips compared to sparsely occupied strips and instead appeared to distribute their eggs among several strips independently of the number of eggs already present. Jehle et al. (2000) recorded the pygmy newt choosing to lay eggs on substrate with eggs already attached but did not specify whether females avoided leaves with more eggs. The results of my study suggest that females may not assess the amount of available substrate present but base their assessment on the quality of the substrate and the ability to effectively wrap the eggs (see 5.5.1). By spreading their egg widely among strips, they may also promote embryo survival through differences in microhabitat conditions.

9.5.4. Implications of findings

Previous studies of oviposition in newts demonstrate non-random site and substrate selection (Miaud, 1995). My experiments support these conclusions and suggest that great crested newts choose oviposition sites which may increase embryo and subsequent larval survival.

9.5.5. Critique of methods

1. These experiments only used artificial egg strips as oviposition substrate. These were chosen since there would be no confounding variables from oxygen production or substances attached to leaves. However in the wild, when faced

with real vegetation, females may exhibit different site selection where a range of biotic and abiotic factors may influence oviposition site selection.

2. This was a small study, only using 30 females in three trials. Larger sample sizes may yield more significant results, especially in relation to whether females actively prefer to lay more eggs on occupied strips.

3. Levels of oxygen were relatively consistent in the tanks due to their shallow depth and constant temperature. In natural ponds where oxygen levels vary, females may avoid laying on a particular substrate, regardless of how many eggs are already attached, due to low oxygen levels in ponds.

9.6. Conclusions

1. Great crested newt females preferred to lay eggs on clean compared to occupied strips. This confirms those findings of other studies that females lay eggs in a non-random fashion. By selecting clean substrate embryo mortality may be lower due to the increased effectiveness of wrapping and decreased risks of infection from fungi and other pathogens.

2. Females will lay eggs on occupied strips possibly to reduce the risks of predation through the dilution effect. However females do not lay fewer eggs on crowded strips perhaps indicating factors affecting larvae may be important in egg location choice. Instead females appear to spread their eggs across many strips.

Chapter 10. Thesis Conclusions

The overall aim of this thesis was to increase our knowledge and understanding of the microhabitat use and ecology of great crested newts within a woodland area in the British Isles and suggest ways of improving conservation measures.

Examination of the population dynamics of adult great crested newts revealed that incidence of capture and detection probabilities fluctuated widely between seasons and ponds highlighting the need for the use of capture-mark-recapture techniques before attaining reliable estimates of population size (Bailey et al., 2004b). Apparent annual adult survival varied by sex but not year or site which is contrary to that found in other studies (e.g. Arntzen & Teunis, 1993; Griffiths et al., 2010) and demonstrates the importance of calculating population-specific values for apparent survival. Further research may seek ways to incorporate capture-mark-recapture techniques into great crested newt surveys leading to more effective assessment of population sizes and identification of priority great crested newt breeding sites.

This study has elucidated some key findings in relation to the terrestrial ecology of juvenile great crested newts, which have previously been unanswered or poorly understood. First, relatively little is known about terrestrial habitat use and movements of juvenile great crested newts (Schabetsberger et al., 2004; Malmgren, 2007). Results from my study indicate that within the first few months or years of life, juveniles may utilise terrestrial cover objects close to natal ponds on a semi-permanent basis whilst occupying a small home range. This highlights the importance of the provision of suitable, undisturbed terrestrial

habitat close to breeding ponds to allow juvenile development. Second, this study added more knowledge to our understanding of the growth rates in juveniles. Findings showed that individuals grew fastest when in the smallest age category (35 to 39 mm), after which growth rates declined with size. Since these individuals were often identified as metamorphs, this suggests that the first year after emerging from ponds may be crucial for growth. This is contrary to other studies (Arntzen, 2000; Sever et al., 2001), which indicated that growth rates slowed later in life. This again highlights the need for location-specific research on great crested newt ecology and demonstrates that extrinsic factors may affect individual growth and maturity. Third, this is the first study to examine the monthly and annual apparent survival of juvenile great crested newts using capture-mark-recapture techniques. Data from this study indicated that juveniles had relatively low apparent annual survival at 0.19. Values of apparent survival were constant with year, indicating that fluctuations in larval recruitment may be more important than variations in juvenile survival in governing population size.

This was the first study to examine the effects of body condition on male crest height in male great crested newts over multiple years and ponds. Results suggest that males with a higher body condition possess larger dorsal crests, especially early in the breeding season. This result was consistent across four years between three ponds. Since males with larger crests may gain more matings (Baker & Halliday, 2000; Hedlund, 1990, Malmgren & Enghag, 2008) these results suggest that crest size may be an honest indicator of body condition. However further research is required to determine whether females actively choose males with larger crests. Also, results suggest that a significant

reduction in body condition index may lead to lowering of average crest height, resulting in less fit, poorer quality males. This in turn could have negative implications on the fitness of larvae and juveniles.

Although the direct negative impacts of predators on great crested newt populations have been well documented (Knapp & Matthews, 2000; Joly et al., 2003) with predators commonly consuming vulnerable larvae no research has been conducted to examine the indirect or non-consumptive effects on great crested newt embryos or larvae. Results from experiments over two years showed increased mortality in great crested newt embryos when raised in the presence of predatory sticklebacks *Gasterosteus aculeatus* compared to controls. This suggests that the impacts of fish may go beyond predation and may give additional explanations as to why great crested newts suffer when in the presence of fish. These experiments were carried out under controlled laboratory conditions so further research is required to determine whether these results occur in field conditions.

In addition, this study found that when faced with caged predatory stickleback larvae utilised vegetated microhabitats for a significantly greater proportion of the time than when in the presence of non-predatory crucian carp *Carassius carassius*. This demonstrates that larvae may be able to exhibit anti-predator responses and determine the level of threat.

The final chapter of this thesis examined the oviposition behaviour of female great crested newts. Although previous studies have examined the egg-laying behaviour of female *Triturus* newts (Miaud,1995; Norris & Hosie, 2005), none have specifically looked at how individuals behave when faced with occupied

compared to clean substrate. Great crested females preferred to lay eggs on clean compared to occupied strips. This demonstrates that females lay eggs in a non-random fashion. By selecting clean substrate embryo mortality may be lower due to increased effectiveness of wrapping and decreased risks of infection from fungi and other pathogens. Therefore female great crested newts demonstrated an ability to make choices about oviposition site selection which may positively affect the fitness and survival of embryos and larvae.

Appendix 1. Habitat Suitability Index

The Habitat Suitability Index (HSI) was devised by Oldam et al., 2000.

Table 1 is adapted and Figures 1 to 3 are reproduced from the original paper:

Oldham, R. S., Keeble, J., Swan, M. J. S. and Jeffcote, M. (2000) Evaluating the suitability of habitat for the great crested newt *Triturus cristatus*. *The Herpetological Journal*, **10**: 143-155.

SI	Factor	Units	Derivation of SI value
1	Location	Measured as map location	Refer to Fig. 2. Zone A: optimal; SI = 1 Zone B: Marginal; SI = 0.5 Zone C: Unsuitable; SI = 0.
2	Pond area	M^2	Measure pond surface area. Read off SI value from Figure 1.
3	Pond	Years	Years out of ten that pond dries during spring/early summer. Read off SI from Figure 1.
4	Water quality	Subjective scale	4 point scale where: 4 = good water quality; water normally clear with abundant and diverse invertebrate community; SI = 1. 3 = moderate water quality; SI = 0.67 2 = poor water quality, low invertebrate diversity; SI = 0.33 1 = bad water quality, clearly polluted with emphasis on species with low oxygen tension; SI = 0.01
5	Shade	%	Estimate% perimeter shoreline shade 1m from shoreline. Read off SI from Figure 1.
6	Fowl	Count	Number of water fowl seen per pond. Read off SI from Figure 1.
7	Fish	Subjective scale	4 point scale where: 4 = absent; SI = 1. 3 = possible; SI = 0.67 2 = minor (e.g. crucian carp, sticklebacks); SI = 0.33. 1 = major (other species of carp); SI = 0.01.
8	Pond	Count	Number of ponds occurring within 1Km of the target (excluding target pond). Use an OS map or field survey. Divide number of ponds by π (=3.14) . Read off SI value from Figure 1.
9	Terrestrial	Map	OX map with 500m radius around pond to indicate 'newt friendly' habitat. Calculate total area of shaded and linear features (A). Multiply this by B (below) and AB is read off the graph in Figure 1. B values: 5 = no serious barrier within 500m; B = 1. 4 = minor barrier e.g. minor road; B = 0.6 3 = moderate barrier e.g. road, river with up to 50% of area difficult to access; B = 0.4. 2 = major barriers with up to 75% of habitat difficult to access; B = 0.6. 1 = almost total barrier to newt movement; B = 0.2.
10	Macroph-yte	%	Estimate% of pond surface area occupied by macrophyte cover (sum of submergent, emergent and floating plants). Estimate using Figure 3 and read off graph from Figure 1.

Table 1. Description of the 10 factors, values and standard index (SI) calculations for the great crested newt habitat suitabliity index.

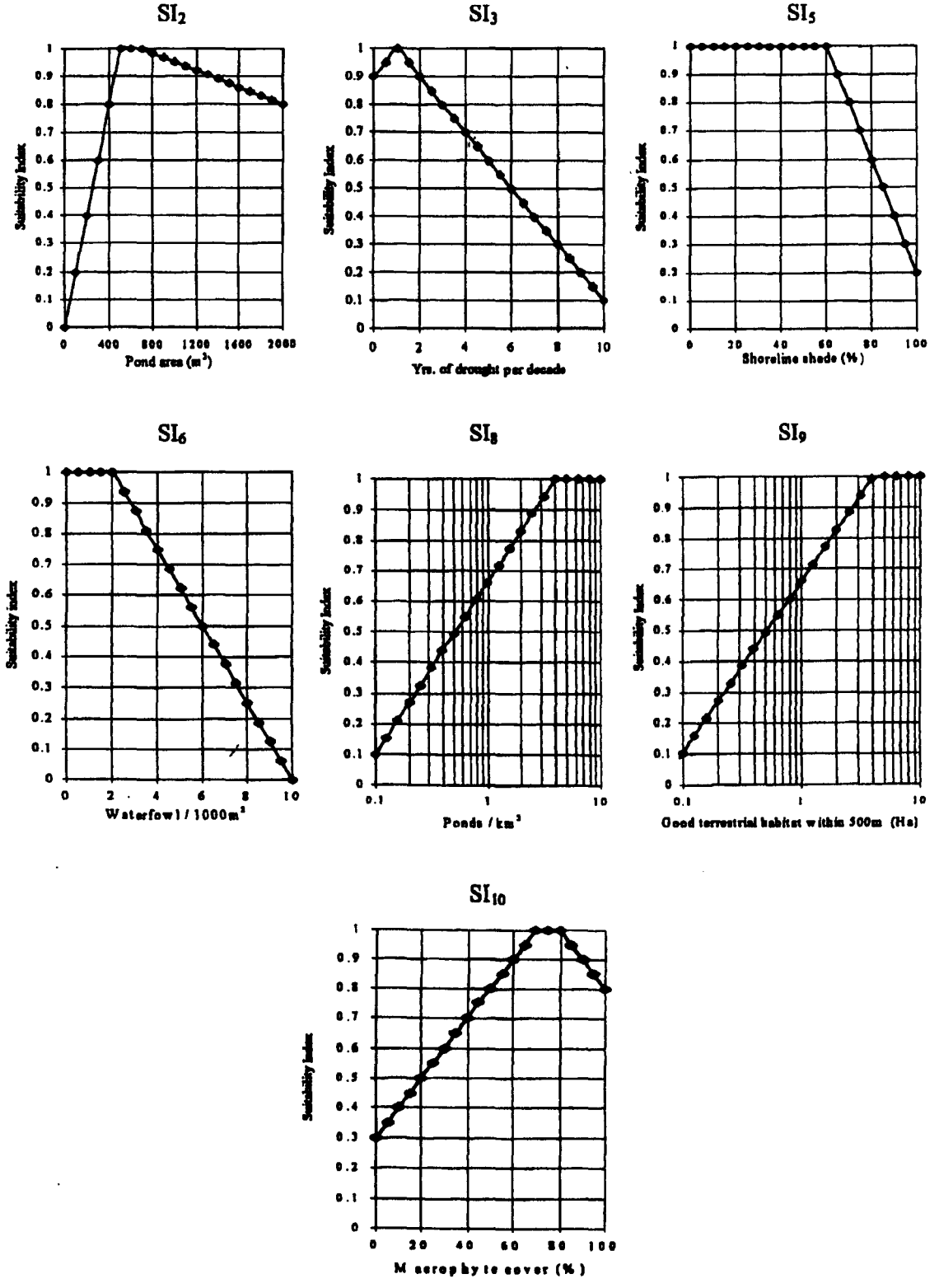


Figure 1. Suitability Index derivation based upon seven separate habitat features.

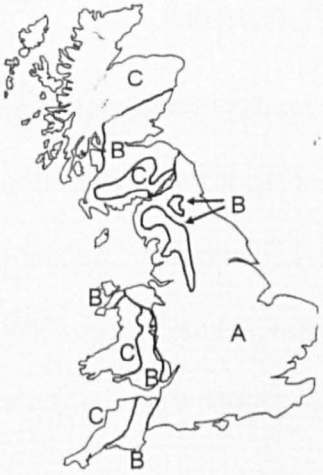


Figure 2. Map used to determine the Suitability Index for location (SI1), based upon the known distribution of the crested newt. Zone A (optimal) has a high probability of crested newt occurrence within each 10 km square; zone B (marginal) with patchy distribution and a low probability of occurrence; zone c (unsuitable) with a very low probability of occurrence, mainly outside the recorded range of the species.

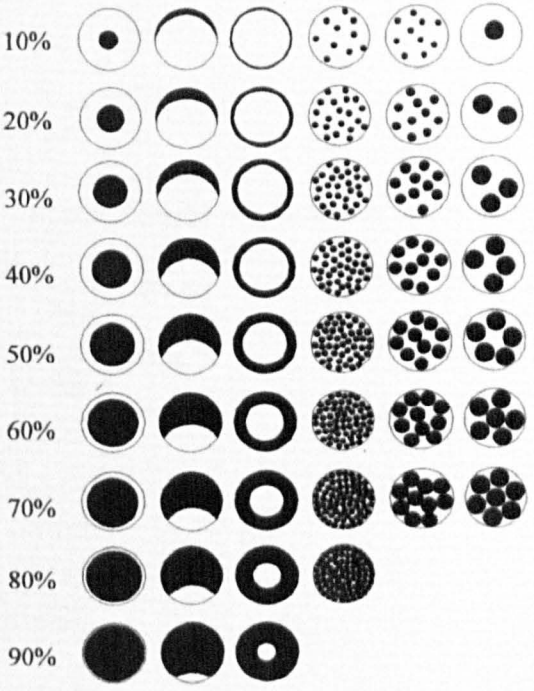


Figure 3. Guide for use in assessment of the proportions of vegetation cover in a pond. The percentage of each circle shaded in the figures is indicated. The circlues simluate a variety of vegetation dispersion patterns.

Appendix 2. Sizes of terrestrial refuges

Each terrestrial refuge was measured with a tape measure to the nearest centimetre. For logs length (L) and circumference (C) and diameter (D) was recorded. Length (L), width (W) and depth (De) of concrete slabs was measured. Stones were flattened, rounded and thus measured as logs. The substrate underneath each refuge was also noted.

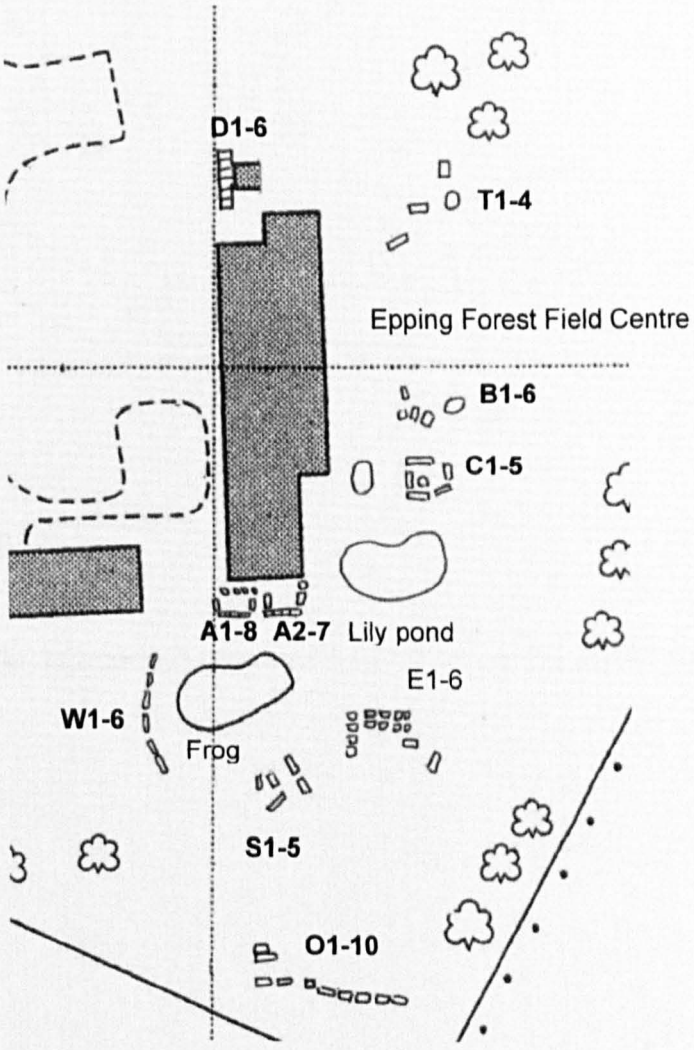
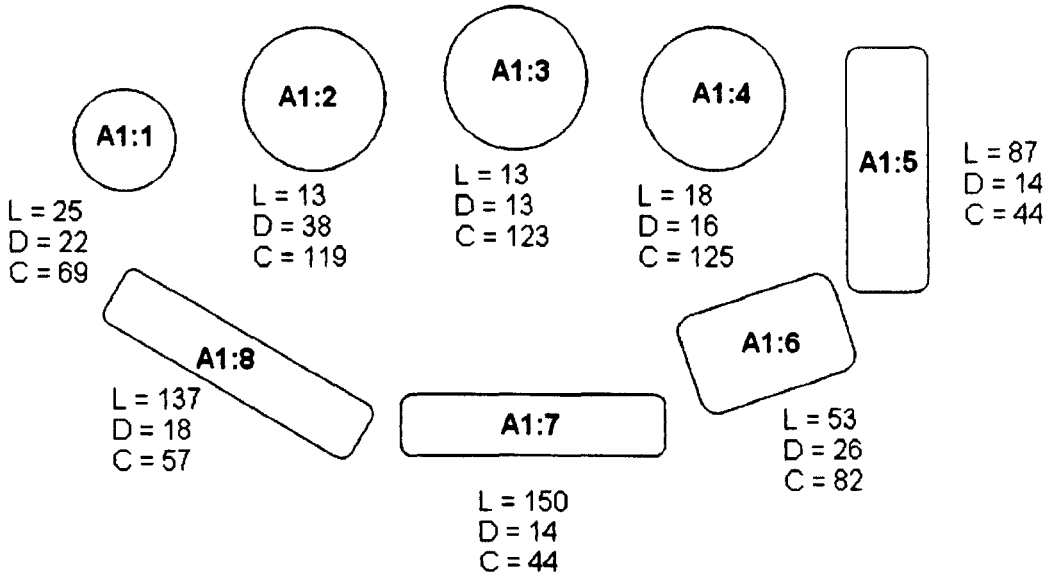


Figure A1. Diagram to show locations of all the terrestrial refuges (see chapter 4).

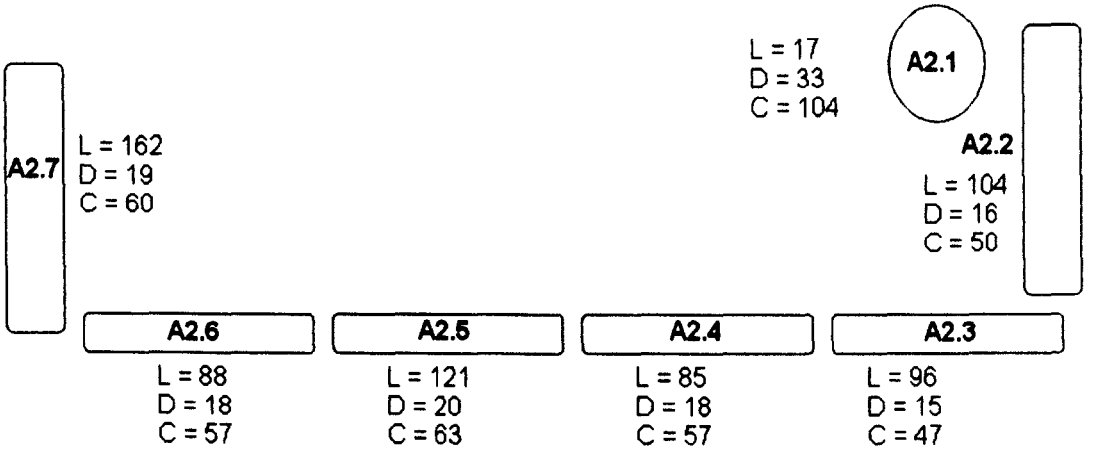
1. Location A1

These logs are situated directly next to the south wall of the Field Centre, 10 m from Frog pond. Four refuges are logs lie on their sides, the remainder stumps flat against the ground. Substrate consists of leaf litter, decaying woodchip and soil.



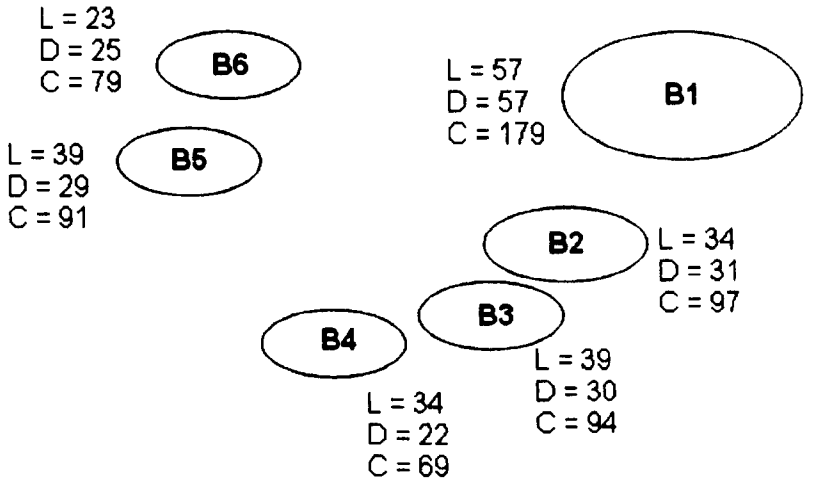
2. Location A2

These 7 refuges lie adjacent to those of A1 and lie on the same substrate type.



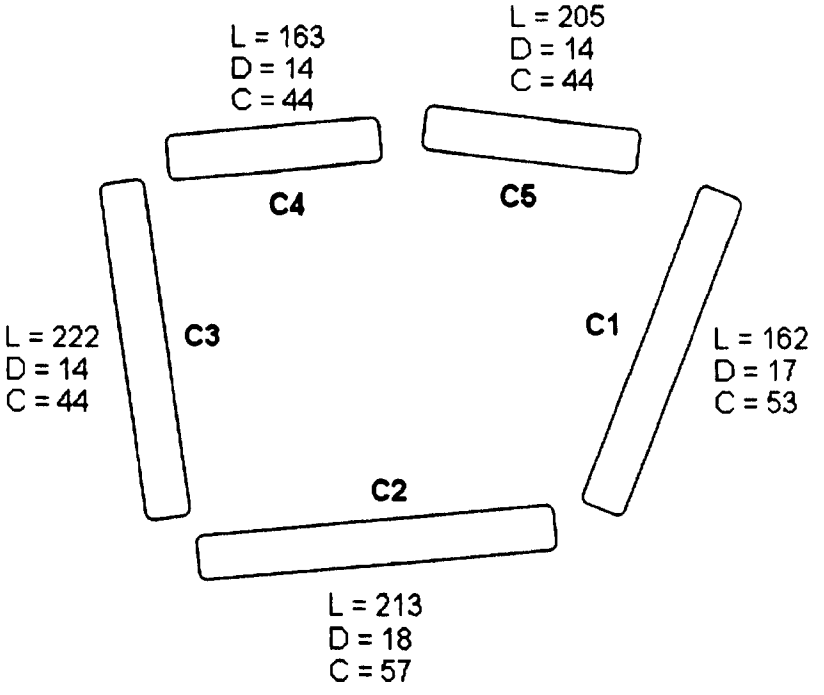
3. Location B

This area lies adjacent to deciduous woodland and scrub. Six refuges consisting of a variety of flattened, rough stones comprise these refuges. All refuges were situated on leaf litter or soil.



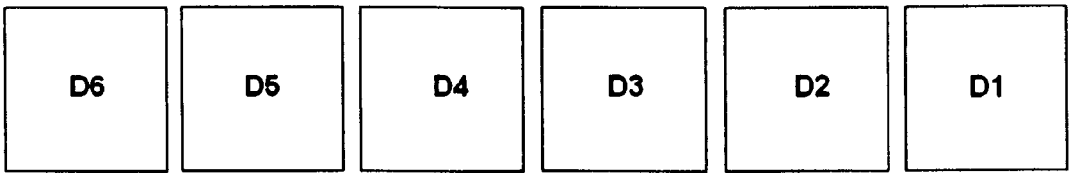
4. Location C

Five long logs located in the centre of a grassy area, surrounded by scrub and deciduous woodland forms this area. All refuges were located on grass and soil.



5. Location D

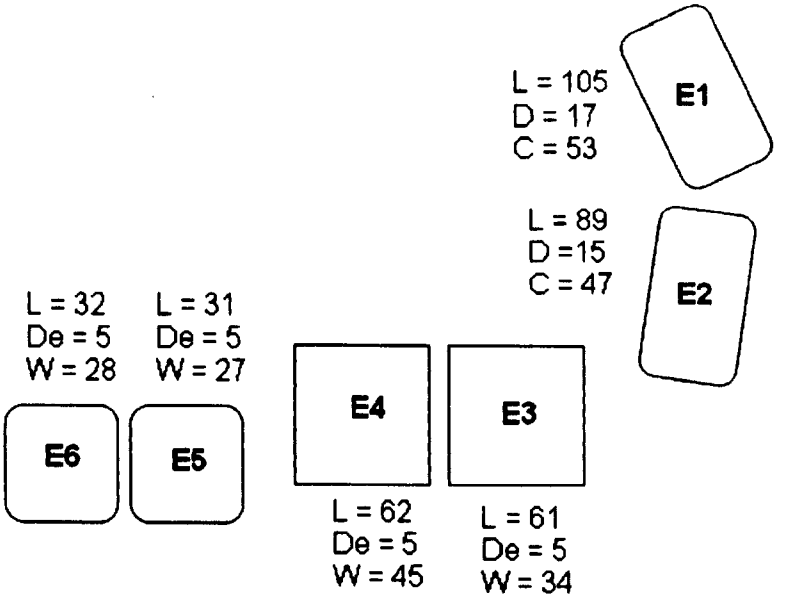
This area consists of six concrete slabs situated next to the Field Centre amongst dense scrub. All were located on damp soil.



All refuges 60 L x 60 W x 5 De

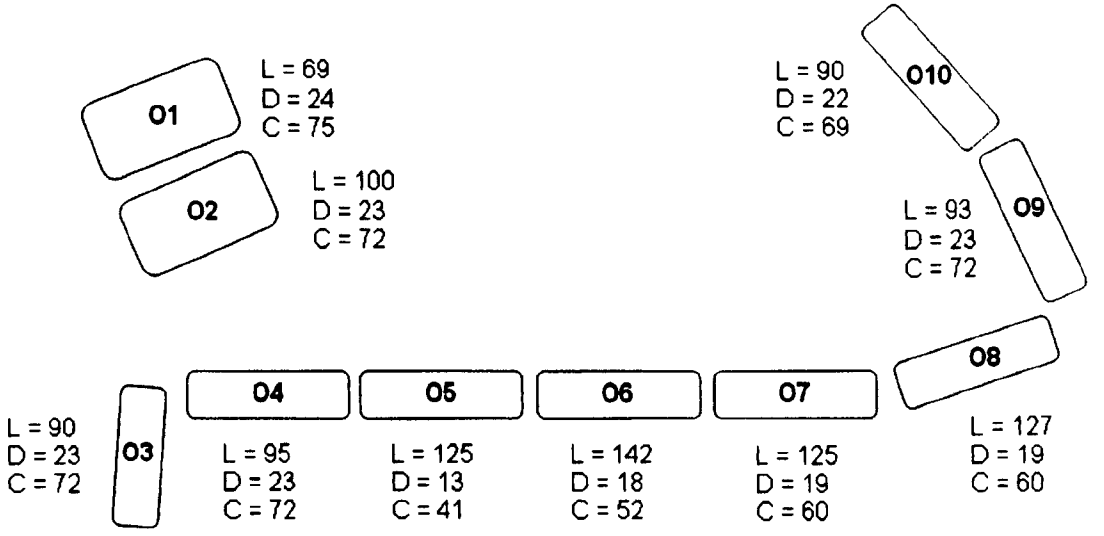
6. Location E

A combination of small concrete slabs and logs made up this area, all located within deciduous woodland. Refuges were placed onto soil and leaf litter.



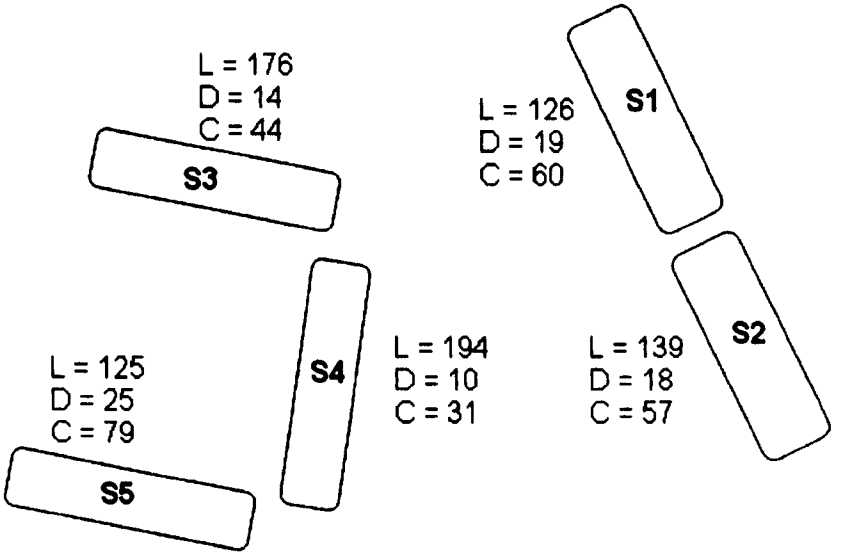
7. Location O

The largest collection of refuges, this area consisted of 10 logs in a row, lining an overgrown footpath within deciduous woodland.



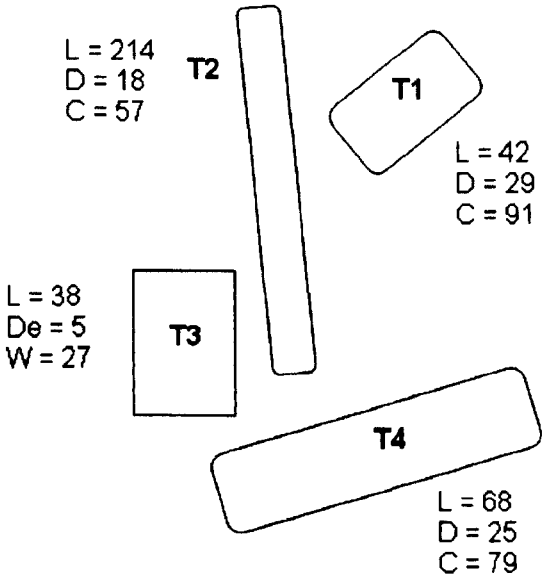
8. Location S

These five logs were located just 5 metres from Frog pond adjacent to deciduous woodland. All were set on soil or leaf litter.



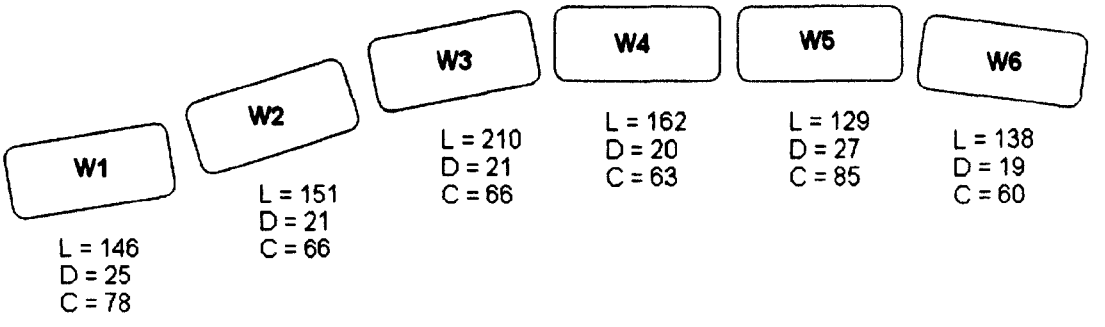
9. Location T

Set in deciduous woodland, adjacent to a field study building, three were logs, one was a concrete slabs. All were placed on either leaf litter or soil.



9. Location W

These refuges were situated 10 m from Frog pond in a line, within scrub and deciduous woodland. All were place on grass and soil.



References

Ahola, M. P., Laaksonen, T., Eeva, T. and Lehikoinen, E. (2009) Great tits increasing smaller clutches than selected for: a study of climate- and density-related change in reproductive traits. *Journal of Animal Ecology*, **78**: 1298-1306.

Amarasekare, P. and Possingham, H. (2001) Patch dynamics and metapopulation theory: the case of successional species. *Journal of Theoretical Biology*, **209**: 333-344.

Alford, R. A. and Richards, S. J. (1999) Global amphibian declines: a problem in applied ecology. *Annual Review of Ecological Systematics*, **30**: 131-165.

Altwegg, R. (2002) Predator-induced life-history plasticity under time constraints in Pool Frogs. *Ecology*, **83** (9): 2542-2551.

AmphibiaWeb (2011) http://amphibiaweb.org/lists/Salamandridae.shtml. Accessed November 2011.

Anderson, M. (1994) *Sexual selection*. Princeton University Press, Princeton, New York.

Anderson, A. R. and Petranka, J. W. (2003) Odonate predator does not affect hatching time or morphology of embryos of two amphibians. *Journal of Herpetology* **37** (1): 65-71.

Andreone, F. (1986) Considerations on marking methods in newts, with particular reference to a variation of the "belly pattern" marking technique. *British Herpetological Society Bulletin*, **16**: 36-37.

Andreone, F. and Giacoma, C. (1989) Breeding dynamics of *Triturus carnifex* at a pond in northwestern Italy (Amphibia, Urodela, Salamandridae). *Holarctic Ecology*, **12** (3): 219-223.

Angelini, C., Antonelli, D. and Utzeri, C. (2010) Capture-mark-recapture analysis reveals survival correlates in *Salamandra perspicillata*. *Amphibia-Reptilia*, **31**: 21-26.

Anholt. B. R., Hotz, H., Guex, G. -D. and Semlitsch, R. D. (2003) Overwinter survival of *Rana lessonae* and its hemiclonal associate *Rana esculenta*. *Ecology*, **84** (2): 391-397.

Anholt, B. R., Werner, E. and Skelly, D. K. (2000) Effect of food and predators on the activity of four larval ranid frogs. *Ecology*, **81** (12): 3509-3521.

Araújo, M. B., Thuiller, W. and Pearson, R. G. (2006) Climate warming and the decline of amphibians and reptiles in Europe. *Journal of Biogeography*, **33**: 1712-1728.

Arntzen, J. W. (2000) A growth curve for the newt *Triturus cristatus*. *Journal of Herpetology*, **34** (2): 227-232.

Arntzen, J. W. (2002) Testing for equal catchability in *Triturus* newts by dip netting. *Journal of Herpetology*, **36** (2): 272-276.

Arntzen, J. W., Smithson, A. and Oldham, R. S. (1999) Marking and tissue sampling effects on body condition and survival in the newt *Triturus cristatus*. *Journal of Herpetology*, **33** (4): 567-576.

Arntzen, J. W. and Teunis, S. F. M. (1993) A six year study on the population dynamics of the crested newt (*Triturus cristatus*) following colonization of a newly created pond. *The Herpetological Journal*, **3** (3): 99-110.

Aronsson, S. and Stenson, J. A. E. (1995) Newt-fish interactions in a small forest lake. *Amphibia-Reptilia*, **16**: 177-184.

Ash, A. N., Bruce, R. C., Castanet, J. and Francillon-Vieillot, H. (2003) Population parameters of *Plethodon metcalfi*, on a 10-year-old clear cut and in nearby forest in the southern Blue Ridge Mountains. *Journal of Herpetology*, **37** (2): 445-452.

Atkins, W. and Herbert, C. (1998) *Amphibian Survey, Epping Forest (Corporation of London), 1998*. (Unpublished).

Azevedo-Ramos, C., Sluys, M. V., Hero, J-M. and Magnusson, W. E. (1992) Influence of tadpole movement on predation by odonate naiads. *Journal of Herpetology*, **26** (3): 335-338.

Baguette, M. (2004) The classical metapopulation theory and the real, natural world: a critical appraisal. *Basic and Applied Ecology*, **5** (4): 213-224.

Bailey, L. L., Simons, T. R. and Pollock, K. H. (2004a) Spatial and temporal variation in detection probability of *Plethodon* salamanders using the robust capture-recapture design. *Journal of Wildlife Management*, **68** (1): 14-24.

Bailey, L. L. Simons, T. R. and Pollock, K. H. (2004b) Estimating detection probability parameters for *Plethodon* salamanders using the robust capture-recapture design. *Journal of Wildlife Management*, **68** (1): 1-13.

Bailey, L. L., Kendall, W. L., Church, D. R. and Wilbur, H. M. (2004c) Estimating survival and breeding probability for pond-breeding amphibians: a modified robust design. *Ecology*, **85** (9): 2456-2466.

Bailey, L. L. Simons, T. R. and Pollock, K. H. (2004d) Estimating site occupancy and species detection probability parameters for terrestrial salamanders. *Ecological Applications*, **14** (3): 692-702.

Bailey, N. W. and Zuk, M., (2008) Acoustic experience shapes female mate choice in field crickets. *Proceedings of the Royal Society, London B,* **275** (1651): 2645-2650.

Baker, J. M. R. (1992) Body condition and tail height in great crested newts, *Triturus cristatus*. *Animal Behaviour*, **43** (1): 157-160.

Baker, J. M. R. (1999) Abundance and survival rates of great crested newts (*Triturus cristatus*) at a pond in central England: monitoring individuals. *Herpetological Journal,* **9**: 1-8.

Baker, J., Beebee, T., Gent, T. and Orchard, D. (2011) Amphibian Habitat Management Handbook. Amphibian and Reptile Conservation, Bournemouth.

Baker, J. M. R. and Halliday, T. R. (1999) Amphibian colonization of new ponds in an agricultural landscape. *Herpetological Journal*, **9**: 55-63.

Baker, J. and Halliday, T. R. (2000) Variation in dorsal crest morphology and tail height with age in great crested newts (*Triturus cristatus*). *Herpetological journal*, **10**: 173-176.

Băncillă, R. I., Hartel, T., Plăiaşu, R., Smuts, J. and Cogălniceanu, D. (2010) Comparing three body condition indices in amphibians: a case study of yellow bellied toad *Bombina variegata*, *Amphibia-Reptilia*, **31**: 558-562.

Banks, B., Beebee, T. J. C. and Cooke, A. S. (1994) Conservation of the natterjack toad *Bufo calamita* in Britain over the period 1970-1990 in relation to site protection and other factors. *Biological Conservation*, **67**: 111-118.

Barks, R. L., Jeppesen, E. and Lodge, D. M. (2000) Macrophyte and fish chemicals suppress *Daphnia* growth and alter life-history traits. *Oikos*, **88** (1): 139-147.

Beattie, R. C., Aston, R. J. and Milner, A. G. P. (1991) A field study of fertilization and embryonic development in the common frog (*Rana temporaria*) with particular reference to acidity and temperature. *Journal of Applied Ecology*, **28** (1): 346-357.

Becker, C. G. and Loyola, R. D. (2008) Extinction risk assessments at the population and species level: implications for amphibian conservation. *Biodiversity Conservation*, **17**: 2297-2304.

Becker, C. G., Fonseca, C. R., Haddad, C. F. B. and Prado, P. I. (2009) Habitat split as a cause of local population declines of amphibians with aquatic larvae. *Conservation Biology*, **24** (1): 287-294.

Beebee, T. J. C. (1973) Observations concerning the decline of the British Amphibia. *Biological Conservation*, **5** (1): 20-24.

Beebee, T. J. C. (1977a) Habitats of the British amphibians (1): Chalk uplands. *Biological*

Conservation, **12**: 279-287.

Beebee, T. J. C. (1977b) Environmental change as a cause of natterjack toad (*Bufo calamita*) declines in Britain. *Biological Conservation*, **11**: 87-102.

Beebee, T. J. C. (1981) Habitats of the British amphibians (4): Agricultural lowlands and a general discussion of requirements. *Biological Conservation*, **21**: 127-139.

Beebee, T. J. C. (1996) *Ecology and Conservation of Amphibians.* Chapman and Hall, London.

Beebee, T. J. C. (1997) Changes in dewpond numbers and amphibian diversity over 20 years on chalk downland in Sussex, England. *Biological Conservation*, **81**, 215-219.

Beebee, T. J. C. (2007) Thirty years of garden ponds. *British Herpetological Bulletin*, **99**: 23-28.

Bell, G. and Lawton, J. H. (1975) The ecology of the eggs and larvae of the smooth newt (*Triturus vulgaris* (Linn.)) *Journal of Animal Ecology*, **44** (2): 393-423.

Bernal, J. A., Page, R. A., Ryan, M. J., Argo, T. F. and Wilson, P. S. (2009) Acoustic radiation patterns of mating calls of túngara frogs (*Physalaemus pustuosus*): implications for multiple receivers. *Journal of Acoustic Society of America*, **126** (5): 2757-2767.

Bernard, M. F. (2004) Predator-induced phenotypic plasticity in organisms with complex life histories. *Annual Review of Ecological Systematics*, **35**: 651-673.

Blackwell, E. A., Cline, G. R. and Marion, K. R. (2004) Annual variation in population estimators for a southern population of *Ambystoma maculatum*. *Herpetologica*, **60** (3): 304-311.

Blaustein, A. R. and Kiesecker, J. M. (2002) Complexity in conservation: lessons from the global decline of amphibian populations. *Ecology Letters*, **5**: 597-608.

Blaustein, A. R., Kiesecker, J. M., Chivers, D. P. and Anthony, R. G. (1997) Ambient UV-B radiation causes deformities in amphibian embryos. *Proceedings of the National Academy of Sciences of the USA*, **94** (25): 13735-13737.

Blaustein, A. R., Hoffman, P. D., Kiesecker, K. M. and Hayes, J. B. (1996) DNA repair activity and resistance to solar UV-B radiation in egg of the red-legged frog. *Conservation Biology*, **10** (5): 1398-1402.

Blaustein, A. R., Hokit, D. G., O'Hara, R. K. and Holt, R. A. (1994a) Pathogenic fungus contributes to amphibian losses in the Pacific northwest. *Biological Conservation*, **67**: 251-254.

Blaustein, A. R., Wake, D. B. and Sousa, W. P. (1994b) Amphibian declines: Judging stability, persistence and susceptibility of populations to local and global extinctions. *Conservation Biology*, **8** (1): 60-71.

Bonato, L. and Fracasso, G. (2003) Movements, distribution pattern and density in a population of *Salamandra atra aurorae* (Caudata: Salamandridae). *Amphibia-Reptilia*, **24**: 251-260.

Bosch, J., Carrascal, L. M., Durán, L., Walker, S. and Fisher, M. C. (2007) Climate change and outbreaks of amphibian chytridiomycosis in a montane area of Central Spain: is there a link? *Proceedings of the Royal Society B*, **274**: 253-260.

Bradford, D. F. (1983) Winterkill, oxygen relations and energy metabolism of a submerged dormant amphibian *Rana muscosa*. *Ecology*, **64** (5): 1171-1183.

Bradford, D. F., Tabatabai, F. and Graber, D. M. (1993) Isolation of remaining populations of the native frog, *Rana muscosa*, by introduced fishes in Sequoia and Kings Canyon National Parks, California. *Conservation Biology*, **7** (4): 882-888.

Breden, F. (1987) The effect of post-metamorphic dispersal on the population genetic structure of Fowler's Toad, *Bufo woodhousei fowleri*. *Copeia*, **1987** (2): 386-395.

Brimble, J. (1991) *Brimble's guide to Epping Forest for ramblers and riders*. Epping Forest Conservation Centre, Loughton, Essex.

Brönmark, C. and Edenhamn, P. (1994) Does the presence of fish affect the distribution of tree frogs (*Hyla arborea*)? *Conservation Biology*, **8** (3): 841-845.

Burek, K. A., Gulland, F. M. D. and O'Hara, T. M. (2008) Effects of climate change on Arctic marine mammal health. *Ecological Applications*, **18** (2): S126-S134.

Burnham, K. P. and Anderson, D. R. (2002) *Model selection and multimodal inference: a practical information-theoretic approach*. 2nd Edition. Springer Science, USA.

Buxton, E. N. (1923) *Epping Forest*. Edward Stainford, London.

Canessa, S., Heard, G. W., Parris, K. M and McCarty, M. A. (2012) Integrating variability in detection probabilities when designing wildlife surveys: a case study of amphibians from south-eastern Australia. *Biodiversity and Conservation*, **21**: 729-744.

Carey, C. and Alexander, M. A. (2003) Climate change and amphibian declines: is there a link? *Diversity and Distributions*, **9**: 111-121.

Castanet, J., Francillon-Vieillot, H. and Bruce, R. C. (1996) Age estimation in Desmognathine salamanders assessed by skeletochronology. *Herpetologica*, **52** (2): 160-171.

City of London (2007) *Epping Forest 2010: The management plan for Epping Forest, 2004-2010*. City of London Corporation, Guildhall, London.

Clemons, J. (1997) Conserving Great Crested Newts. *British Society of Herpetology Bulletin*, **59**: 2-5.

Clutton-Brock, T. (2007) Sexual selection in males and females. *Science*, **318** (5858): 1882-1885.

Collins, J. P. and Storfer, A. (2003) Global amphibian declines: sorting the hypotheses. *Diversity and Distributions*, **9**: 89-98.

Cooke, A. S. (1986) The warty newt *Triturus cristatus* at Shillow Hill: ranging on arable land. *Report of Huntingdon Flora Society*, **38**: 40-44.

Cooke, A. S. (1994) Fluctuations in night counts of crested newts at eight breeding sites in Huntingdonshire 1986-1993. In: *Conservation and management of great crested newts: proceedings of a symposium held on 11th January 1994 at Kew gardens, Richmond, Surrey.* Eds T. Gent and R. Bray. English Nature science report No. 20. English Nature, Peterborough.

Cooke, A. S. (1995) A comparison of survey methods for crested newts (*Triturus cristatus*) and night counts at a secure site, 1983-1993. *Herpetological Journal*, **5**: 221-228.

Cooke, A. S. and Arnold, H. R. (2003) Night counting, netting and population dynamics of crested newts (*Triturus cristatus*). *Herpetological Bulletin*, **84**: 5-14.

Cooke, A. S. and Scorgie, H. R. A. (1983) *The status of the commoner amphibians and reptiles in Britain.* Nature Conservancy Council, Report No. 3, Shrewsbury.

Corbett, K. (1994) European perspective and status. In: *Conservation and management of great crested newts: proceedings of a symposium held on 11th January 1994 at Kew gardens, Richmond, Surrey.* Eds T. Gent and R. Bray. English Nature science report No. 20. English Nature, Peterborough.

Corporation of London (2000) *Annual report for the Superintendent for 1999/00.* Corporation of London, Guildhall, London.

Corporation of London (2002) *Annual report for the Superintendent for 2001/02.* Corporation of London, Guildhall, London.

Crochet, P-A., Chaline, O., Cheylan, M. and Guillaume, C. P. (2004) No evidence of general decline in amphibian community in southern France. *Biological Conservation*, **119**: 297-304.

Curando, N., Hartel, T. and Arntzen, J. W. (2011) Amphibian pond loss as a function of landscape change – a case study over three decades in an agricultural area of northern France. *Biological Conservation*, **144**: 1610-1618.

Cushman, S. A. (2006) Effects of habitat loss and fragmentation on amphibians: a review and

prospectus. *Biological Conservation*, **128**: 231-240.

Darwin, C. (1871) *The descent of man, and selection in relation to sex.* John Murray, London.

D'Amen, M. and Bombi, P. (2009) Global warming and biodiversity: evidence of climate-linked amphibian declines in Italy. *Biological Conservation*, **142**: 3060-3067.

Daszak, P., Cunningham, A. A. and Hyatt, A. D. (2003) Infectious disease and amphibian population declines. *Diversity and Distributions*, **9**: 141-150.

Defra (2009) *Adapting to climate change: UK climate projections*. Department for Environment, Food and Rural Affairs, London.

Denöel, M., Whiteman, H. H. and Wissinger, S. A. (2007) Foraging tactics in alternative heterochromic salamander morphs: trophies quality of ponds matters more than water permanency. *Freshwater Biology*, **52**: 1667-1676.

DeWitt, T. J., Sih, A. and Wilson, D. S. (1998) Costs and limits of phenotypic plasticity. *Trends in Ecology and Evolution*, **13** (2): 77-81.

Dijkstra, P. D., Hemelrijk, C., Seehaursen, O. and Groothuis, T. G. G. (2009) Color polymorphism and intrasexual competition in assemblages of cichlid fish. *Behavioral Ecology*, **20** (1): 138-144.

Dillon, M. E. and Fiaño, J. (2000) Oviposition site selection by the Túngara Frog (*Physalaemus pustulosus*). *Copeia*, **2000** (3): 883-885.

Di Minin, E. and Griffiths, R. A. (2011) Viability analysis of a threatened amphibian population: modelling the past, present and future. *Ecology*, **34** (1): 162-169.

Dodd Jr. C. K. and Dorazio, R. M. (2004) Using counts to simultaneously estimate abundance and detection probabilities in a salamander community. *Herpetologica*, **60** (4): 468-478.

Dolmen, D. (1983) Diel rhythms and microhabitat preferences of the newts *Triturus vulagris* and T. *cristatus* at the northern border of their distribution area. *Journal of Herpetology*. **17** (1): 23-31.

Duellman, W. E. and Trueb, L. (1986) *Biology of Amphibians*, McGraw-Hill Publishing Company, United States of America.

English Nature (2001a) *Great crested newt mitigation guidelines. Version: August 2001*. English Nature, Peterborough, England.

English Nature (2001b) *Eradication of sticklebacks from Lyppard Grange SSSI for the benefit of breeding Great Crested Newts* (unpublished). Report to English Nature Biodiversity Grant Scheme.

Fasola, M. and Canova, L. (1992) Residence in water by the newts *Triturus vulgaris*, *T cristatus* and *T. alpestris* in a pond in northern Italy. *Amphibia-Reptilia*, **13**: 227-233.

Fernández-Benéitez, M. J., Ortiz-Santaliestra, M. E., Lizana, M. and Diéguez-Uribeondo, J. (2008) *Saprolegnia diclina*: another species responsible for the emergent disease '*Saprolegnia* infections' in amphibians. *Federation of European Microbiological Sciences Letters*, **279**: 23-29.

Ferrari, M. C. O. and Chivers, D. P. (2009) Sophisticated early life lessons: threat- sensitive generalisation of predator recognition by embryonic amphibians. *Behavioral Ecology*, **20**: 1295-1298.

Ficetola, G. F. and De Bernardi, F. (2004) Amphibians in a human dominated landscape: the community structure is related to habitat features and isolation. *Biological Conservation*, **119** (2): 219-230.

Fisher, R. A. (1930) *A genetical theory of natural selection*. 2nd Edition. Dover, New York.

Forester, D. C., Snodgrass, J. W., Marsalek, K., and Kanham, Z. (2006) Post-breeding dispersal and summer home range of female American Toads (*Bufo americanus*). *North Eastern Naturalist*, **13** (1): 59-72.

Forsman, A. and Hagman, M. (2006) Calling is an honest indicator of paternal genetic quality in poison frogs. *Evolution*, **60** (10): 2148-2157.

Francillon-Vieillot, H., Arntzen, J. W. and Géraudie, J. (1990) Age, growth and longevity of sympatric *Triturus cristatus*, *T. marmoratus* and their hybrids (Amphibia, Urodela): A skeletochronological comparison. *Journal of Herpetology*, **24** (1): 13-22.

Gabor, C. R. and Halliday, T. R. (1997) Sequential mate choice by multiply mating smooth newts: females become more choosy. *Behavioral Ecology*, **8** (2): 162-166.

Gabor, C. R., Krenz, J. D. and Jaeger. R. G. (2000) Female choice, male interference, and sperm precedence in the red-spotted newt. *Behavioral Ecology*, **11** (1): 115-124.

Gallien, L. and Bidaud, O. (1959) Table chronoloquie du développement chez *Triturus helveticus* Razoumowsky. *Bulletin of the Zoological Society*, France LXXXIV, No. 1. 22-32.

Gamble. L. R., McGarigal, K., Sigourney, D. B. and Timm, B. C. (2009) Survival and breeding frequency in marbled salamanders (*Ambystoma opacum*): implications for spatio-temporal population dynamics. *Copeia*, **2009** (2): 394-407.

Gamble, L., Ravela, S. and McGarigal, K. (2008) Multi-scale features for identifying individuals

in large biological databases: an application of pattern recognition technology to the marbled salamander *Ambystoma opacum*. *Journal of Applied Ecology*, **45**: 170-185.

Gamradt, S. C. and Kats, L. B. (1996) Effect of introduced crayfish and mosquito fish on Californian newts. *Conservation Biology*, **10** (4): 1155-1162.

Gardner, T. A., Barlow, J. and Peres, C. A. (2007) Paradox, presumption and pitfalls in conservation biology: the importance of habitat change for amphibians and reptiles. *Biological Conservation*, **138**: 166-179.

Gibbons, M. M. and McCarthy, T. K. (1984) Growth, maturation and survival of frogs *Rana temporaria* L., *Holarctic Ecology*, **7** (4): 419-427.

Gill, D. E. (1978) The metapopulation ecology of the red-spotted newt, *Notophthalamus viridescens* Raffinesque, *Ecological Monographs*, **48** (2): 145-166.

Girardello, M., Griggio, M., Whittingham, M. J., and Rushton, S. P. (2010) Models of climate associations and distributions of amphibians in Italy. *Ecological Resolutions*, **25**: 103-111.

Glandt, D. (1982) Abundanzmessungen an mitteleuropäischen *Triturus* – populationen (Amphibia, Salamandridae). *Amphibia-Reptilia*, **4**: 317-326.

Goldberg, F. J., Quinzio, S. and Vaira, M. (2006) Oviposition-site selection by the toad *Melanophryniscus rubriventris* in an unpredictable environment in Argentina. *Canadian Journal of Zoology*, **84**: 699-705.

Gomez, D., Richardson. C., Lengagne, T., Plenet, S., Joly, P., Jean-Paul, L. and Théry, M. (2009) The role of nocturnal vision in mate choice: females prefer conspicuous males in the European tree frog (*Hyla arborea*). *Proceedings of the Royal Society, London B*, **267** (1666): 2351-2358.

Gomez-Mestre, I. Wiens, J. J. and Warkentin, K. M. (2008) Evolution of adaptive plasticity: risk sensitive hatching in neotropical leaf-breeding tree frogs. *Ecological Monographs*, **78** (2): 205-224.

Green, A. J. (1991) Large male crests, an honest indicator of condition, are preferred by smooth newts, *Triturus vulgaris* (Salamandridae) at the spermatophore transfer stage. *Animal Behaviour*, **41** (2): 367-369.

Green, A. J. (1999) Implications of pathogenic fungi for life-history evolution in amphibians. *Functional Ecology*, **13** (4): 573-575.

Green, A. J. (2001) Mass/length residuals: measures of body condition or generators of

spurious results? *Ecology*, **82** (5): 1473-1483.

Greene, A. E. and Funk, W. C. (2009) Sexual Selection on morphology in an explosive breeding amphibian, the Columbia Spotted Frog (*Rana luteiventris*). *Journal of Herpetology*, **43** (2): 244-251.

Griffis-Kyle, K. L. and Ritchie, M. E. (2007) Amphibian survival, growth and development in response to mineral nitrogen exposure and predator cues in the field: an experimental approach. *Oecologia*, **152**: 633-642.

Griffiths, R. A. (1984) Seasonal behaviour and intrahabitat movements in an urban population of smooth newts, *Triturus vulgaris* (Amphibia: Salamandridae). *Journal of Zoology, London*, **203**: 241-251.

Griffiths, R.A. (1985) A simple funnel trap for studying newt populations and an evaluation of trap behaviour in smooth and palmate newts, *Triturus vulgaris* and *T. helveticus*. *Herpetological Journal*, **1**: 5-10.

Griffiths, R. A. (1986) Feeding niche overlap and food selection in smooth and palmate newts, *Triturus vulgaris* and T. *helveticus*, at a pond in mid-Wales. *Journal of Animal Ecology*, **55**: 501-214.

Griffiths, R. A. (1993) The effect of pH on the feeding behaviour in newt larvae (*Triturus*: Amphibia). *Journal of Zoology, London*, **231**: 285-290.

Griffiths, R. A. (1996) *Newts and salamanders of Europe*. Academic Press, London.

Griffiths, R. A. (1997) Temporary ponds as amphibian habitats. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **7**: 119-126.

Griffiths, R. A. (2001) The crested newt (*Triturus cristatus*): Distribution, biology, ecology and protection: 11-12 November, 2000, Rostock, Germany. *Froglog*, **44** (2).

Griffiths, R. A. and Inns, H. (2003) Surveying. In: *Herpetofauna workers' manual*. Eds: A. H. Gent and S. D. Gibson, Peterborough, Joint Nature Conservation Committee.

Griffiths, R. A. and Langton, T. (2003) Catching and Handling. In: *Herpetofauna workers' manual*. Eds: A. H. Gent and S. D. Gibson, Peterborough, Joint Nature Conservation Committee.

Griffiths, R. A. and Mylotte, V. J. (1987) Microhabitat selection and feeding relations of smooth and warty newts, *Triturus vulgaris*, and *T. cristatus*, at an upland pond in mid-Wales. *Holarctic Ecology*, **10** (1): 1-7.

Griffiths, R. A. and Mylotte, V. J. (1988) Observations on the development of the secondary

sexual characters of male newts, *Triturus vulgaris* and T. *helveticus*. *Journal of Herpetology*, **22** (4): 476-480.

Griffiths, R. A., Sewell, D. and McCrea, R. (2010) Dynamics of a declining amphibian metapopulation: survival, dispersal and the impact of climate. *Biological Conservation*, **143**: 485-491.

Griffiths, R. A. and Williams, C. (2000) Modelling population dynamics of the great crested newt (*Triturus cristatus*): a population viability analysis. *The Herpetological Journal*, **10**: 157-164.

Grist, E. P. M. (1994) Climatic factors affecting the activity of natterjack (*Bufo calamita*) and common toads (*Bufo bufo*) outside the breeding season: Mathias revisited. *Herpetological Journal*, **4**: 126-131.

Gustafson, D. H., Pettersson, C. J. and Malmgren, J. C. (2007) Great crested newts (*Triturus cristatus*) as indicators of aquatic plant diversity. *Herpetological Journal*, **16** (4): 347-352.

Haerty, W., Gentilhomme, E. and Secondi, J. (2007) Female preference for a male sexual trait uncorrelated with male body size in the palmate newt (*Triturus helveticus*), *Behaviour*, **144** (18): 797-814.

Hagström, T. (1979) Population ecology of *Triturus cristatus* and *T. vulgaris* (Urodela) in SW Sweden, *Holarctic Ecology*, **2** (2): 108-114.

Halley, J. M., Oldham, R. S. and Arntzen, J. W. (1996) Predicting the persistence of amphibian populations with the help of a spatial model. *The Journal of Applied Ecology*, **33** (3): 455-470.

Halliday, T. R. (1978) Sexual selection and mate choice. Pages 180-213. *In:* J. R. Krebs and N. B. Davis, eds. *Behavioural ecology: an evolutionary approach*. Blackwell, Oxford.

Halliday, T. R. and Tejedo, M. (1995) Intrasexual selection and alternative mating behaviour. In: *Amphibian Biology, volume 2*. Pages 419-468. Eds H. Heatwole and B. K. Sullivan, Surrey Beatty and Sons, Chipping Norton, Australia.

Halliday, T. R. and Verrell, P. A. (1988) Body size and age in amphibians and reptiles. *Journal of Herpetology*, **22** (3): 253-265.

Hamar, A. J. and McDonnell, M. J. (2008) Amphibian ecology and conservation in the urbanising world: a review. *Biological Conservation*, **141** (10): 2432-2449.

Hamilton, D. and Zuk, M. (1982) Heritable true fitness and bright birds: a role for parasites?

Science (new series), **218** (4570): 384-387.

Hampton, P. (2007) A comparison of the success of artificial cover types for capturing amphibians and reptiles. *Amphibia-Reptilia*, **28** (2007): 433-437.

Hanski, I. (1994) A practical model of metapopulation dynamics. *Journal of Animal Ecology*, **63** (1): 151-162.

Hanski, I. (1998) Metapopulation dynamics. *Nature*, **396** (5 Nov): 41-49.

Hanski, I. and Gyllenberg, M. (1993) Two general metapopulation models and the core-satellite species hypothesis. *The American Naturalist*, **142** (1): 17-41.

Hanski, I., Kuussaari, M. and Nieminen, M. (1994) Metapopulation structure and migration in the butterfly *Melitaea cinxia*. *Ecology*, **75** (3): 747-762.

Harrison, J. D. (1987) Food and feeding relations of common frog and common toad tadpoles (*Rana temporaria* and *Bufo bufo*) at a pond in mid-Wales. *Herpetological Journal*, **1**: 141-143.

Hayward, R. (2010) Dispersion and orientation in newly metamorphosed great crested newts (*Triturus cristatus*). PhD thesis, DeMontford University.

Hayward, R., Oldham, R. S., Watt, P. J. and Head, S. M. (2000) Dispersion patterns of young great crested newts (*Triturus cristatus*). *Herpetological Journal*, **10**: 129-136.

Hecnar, S. J. and M'Closkey, R. T. (1997) The effects of predatory fish on amphibian species richness and distribution. *Biological Conservation*, **79**: 123-131.

Hedlund, L. (1990) Reproductive ecology of crested newts *Triturus cristatus* (Laurenti). PhD thesis, Department of Wildlife Ecology, Swedish University of Agricultural Sciences.

Hedlund, L. and Robertson, J. G. M. (1990) Lekking behaviour in crested newts, *Triturus cristatus*. *Ethology*, **80**: 111-119.

Hels, T. and Nachman, G. (2002) Simulating viability of a spadefoot toad *Pelobates fuscus* metapopulation in a landscape fragmented by a road. *Ecography*, **25**: 730-744.

Hemelaar, A. (1988) Age, growth and other population characters of *Bufo bufo* from different latitudes and altitudes. *Journal of Herpetology*, **22** (4): 369-388.

Hoem, S. A., Melis, C., Linnell, J. D. C. and Andersen, R. (2007) Fighting behaviour in territorial male roe deer *Capreolus capreolus*: the effects of antler size and residence. *European Journal of Wildlife Research*, **55** (1): 1-8.

Hoefler, C. D., Persons, M. H. and Rypstra, A. L. (2008) Evolutionarily costly courtship displays in a wolf spider: a test of viability indicator theory. *Behavioral Ecology*, **19** (5): 974-979.

Hokit, D. G., Stith, B. M. and Branch, L. C. (2001) Comparison of two types of metapopulation models in real and artificial landscapes. *Conservation Biology*, **15** (4): 1102-1113.

Holenweg, A. -K and Reyer, H. -U. (2000) Hibernation behavior of *Rana lessonae* and *R. esculenta* in their natural habitat. *Oecologia*, **123**: 41-47.

Homan, R. H., Reed, J. M. and Windmiller, B. S. (2003) Analysis of spotted salamander (*Ambystoma maculatum*) growth rates based on long-bone growth rings. *Journal of Herpetology*, **37** (3): 617-621.

Horner, H. A. and Macgregor, H. C. (1985) Normal development in newts (*Triturus*) and its arrest as a consequence of an unusual chromosome situation. *Journal of Herpetology* **19** (2): 261-270.

Horton, P. J. and Branscombe, J. (1994) Case study: Lomax Brow: great crested newt project. In: *Conservation and management of great crested newts: proceedings of a symposium held on 11th January 1994 at Kew gardens, Richmond, Surrey*. Eds T. Gent and R. Bray. English Nature science report No. 20. English Nature, Peterborough.

Houlahan, J. E., Findlay, C. S., Schmidt, B. R., Meyer, A. H. and Kuzmin, S. L. (2000) Quantitative evidence for global amphibian population declines. *Nature*, **404** (13 April 2000): 752-755.

Hyde, E. J., and Simons, T. R. (2001) Sampling plethodontid salamanders: sources of variability. *Journal of Wildlife Management*, **65** (4): 624-632.

Ireland, D. H., Wirsing, A. J. and Murray, D. L. (2007) Phenotypically plastic responses of green frog embryos to conflicting predation risk. *Oecologia*, **152**: 162-168.

IUCN (2011) http://www.iucn.org/what/tpas/biodiversity/about/biodiversity_crisis. Accessed 22 November, 2011

IUCN (2011a) http://www.iucn.org/about/union/secretariat/offices/europe/about/european_biodiversity/. Accessed 22 November, 2011.

Iwai, N., Kagaya, T. and Okochi, I. (2007) Choice of oviposition site by *Rana japonica*: role of the developmental stage of conspecific eggs. *Herpetologica*, **63** (1): 31-34.

Jahn, P. (1995) Untersuchungen zur Populationsökologie von *Triturus cristtus* (Laurenti, 1768) und *T. vulgaris*, (Linnaeus, 1758) am Friedeholzer Schlatt. Diplomarbeit Universität Bremen, unpublished.

Jakob, E. M., Marshall, S. D. and Uetz, G. W. (1996) Estimating fitness: a comparison of body condition indices. *Oikos*, **77** (1): 61-67.

Jakob, C., Seitz, A., Crivelli, A. J. and Miaud, C. (2002) Growth cycle of the marbled newt *Triturus marmoratus* in the Mediterranean region assess by skeletochronology. *Amphibia-Reptilia*, **23** (4): 407-418.

Jalilpoor, J., Masouleh, A. S. and Masoumzadeh, M. (2006) Fungal flora in *Acipenser persicus* eggs with particular emphasis on *Saprolegnia* sp. (Oomycetes) and mortality during mass incubation at Shahid Beheshti hatchery. *Journal of Applied Ichthyology*, **22** (suppl. 1): 265-268.

Janin, A., Léna, J.-P. and Joly, P. (2011) Beyond occurrence: body condition and stress hormone as integrative indicators of habitat availability and fragmentation in the common toad. *Biological Conservation*, **144** (3): 1008-1016.

Jehle, R. (2000) The terrestrial summer habitat of radio-tracked great crested newts (*Triturus cristatus*) and marbled newts (*Triturus marmoratus*). *Herpetological Journal*, **10**: 143-156.

Jehle, R. and Arntzen, J. W. (2000) Post-breeding migrations of newts (*Triturus cristatus*) and (*T. marmoratus*) with contrasting ecological requirements. *Journal of Zoology, London*, **251**: 297-306.

Jehle, R., Bouma, P., Sztatacsny, M. and Arntzen, J. W. (2000) High aquatic niche overlap in the newts *Triturus cristatus* and *T. marmoratus* (Amphibia, Urodela). *Hydrobiologia*, **437**: 149-155.

Jehle, R., Thiesmeier, B., and Foster, J. (2011) *The Crested Newt: a dwindling pond-dweller*. Laurenti-Verlag, Bielefeld, Germany.

Johnson, T. W., Seymour, R. L. and Padgett, D. E. (2002) *Biology and systematics of the Saprolegniaceae*. On-line publication: http://dl.uncw.edu/digilib/biology/fungi/taxonomy%and%20systematics/padgett%20book. Accessed 25/06/2008.

Joly. P. Miaud, C., Lehmann, A. and Grolet, O. (2001) Habitat matrix effects on pond occupancy in newts. *Conservation Biology*, **15** (1): 239-248.

Joly, P., Morand, C. and Cohas, A. (2003) Habitat fragmentation and amphibian conservation: building a tool for assessing landscape matrix connectivity. *Comptes Rendus Biologies*, **326** (Suppl. 1): 132-139.

Jordão, L. C. and Volpato, G. L. (2000) Chemical transfer of warning information in non-injured fish. *Behaviour*, **137** (6): 681-690.

Kaplan, R. H. and Sherman, P. W. (1980) Intraspecific oophagy in Californian newts. *Journal of*

Herpetology, **14** (2): 183-185.

Kats, L. B. and Ferrer, R. P. (2003) Alien predators and amphibian declines: review of two decades of science and the transition to conservation. *Diversity and Distributions*, **9**: 99-110.

Kendall, W. L., Nichols, J. D. and Hines, J. E. (1997) Estimating temporary emigration using capture-recapture data with Pollock's Robust Design. *Ecology*, **78** (2): 563-578.

Khonsue, W., Matsui, M., Hirai, T. and Misawa, Y. (2001) A comparison of age structures in two populations of the pond frog *Rana nigromaculata* (Amphibia: Anura). *Zoological Science*, **18**: 597-603.

Kiesecker, J. M. and Blaustein, A. R. (1995) Synergism between UV-B radiation and pathogen magnifies amphibian embryo mortality in nature. *Proceedings of the National Academy of Sciences of the United States of America*, **92**: 11049-11053.

Kiesecker, J. M. and Blaustein, A. R. (1997) Influences of egg-laying behaviour on pathogenic infection of amphibian eggs. *Conservation Biology*, **11** (1): 214-220.

Kiesecker, J .M. and Blaustein, A. R. (1999) Pathogen reverses competition between larval amphibians. *Ecology*, **80** (7): 2442-2448.

Kiesecker, J. M., Blaustein, A. R. and Miller, C. L. (2001) Transfer of a pathogen from fish to amphibians. *Conservation Biology*, **15** (4): 1064-1070.

Kinkead, K. E. and Otis, D. L. (2007) Estimating superpopulation size and annual probability of breeding for pond-breeding salamanders. *Herpetologica*, **63** (2): 151-162.

Kleeberger, S. R. and Werner, J. K. (1983) Post-breeding migration and summer movement of *Ambystoma maculatum*. *Journal of Herpetology*, **17** (2): 176-177.

Kloskowski, J. (2009) Size structured effects of common carp on reproduction of pond-breeding amphibians. *Hydrobiologia*, **635**: 205-213.

Knapp, R. A. and Matthews, K. R. (2000) Non-native fish introductions and the decline of the mountain yellow-legged frog from within protected areas. *Conservation Biology*, **14** (2): 428-438

Kodrick-Brown, A. and Brown, J. H. (1984) Truth in advertising: the kinds of traits favored by sexual selection. *The American Naturalist*, **124** (3): 309-323.

Kopecký, O., Vojar, J. and Denoël, M. (2010) Movements of Alpine newts (*Mesotriton alpestris*) between small aquatic habitats (ruts) during the breeding season. *Amphibia-Reptilia*, **31**: 109-116.

Kupfer, A. and Kneitz, S. (2000) Population ecology of the great crested newt (*Triturus cristatus*) in an agricultural landscape: dynamics, pond fidelity and dispersal. *Herpetological Journal*, **10** (4): 165-172.

Laan, R. and Verboom, B. (1990) Effects of pool size and isolation on amphibian communities. *Biological Conservation*, **54**: 251-262.

La Marca, E., Lips, K. R., Lötters, S., Puschendorf, R., Ibáñez, R., Rueda-Almonacid, J. V., Schulte, R., Marty, C., Castro, F., Manzanilla-Puppo, J., García-Pérez, J. E., Bolaños, F., Chaves, G., Pounds, A. J., Toral, E. and Young, B. E. (2005) Catastrophic population declines and extinctions in Neotropical harlequin frogs (Bufonidae: *Atelopus*). *Biotropica*, **37** (2): 190-201.

Langton, T. E. S., Beckett, C. L. and Foster, J. P. (2001). *Great Crested Newt Conservation Handbook*, Froglife, Halesworth.

Lass, S. and Bittner, K. (2002) Facing multiple enemies: parasitized hosts respond to predator kairomones. *Oecologia* **132** (3): 344-349.

Lass, S., Vos, M., Wolinska, J. and Spaak, P. (2005) Hatching with the enemy: *Daphnia* diapausing eggs hatch in the presence of fish kairomones. *Chemoecology*, **15**: 7-12.

Latham, D. M. and Oldham, R. S. (1996) Woodland management and conservation of the great crested newt (*Triturus cristatus*). *Aspects of Applied Biology*, **44**: 451-459.

Laurila, A. and Aho, T. (1997) Do female common frogs choose their breeding habitat to avoid predation on tadpoles? *Oikos*, **78** (3): 585-591.

Laurila, A. and Kujasalo, J. (1999) Habitat duration, predation risk and phenotypic plasticity in common frog (*Rana temporaria*) tadpoles. *Journal of Animal Ecology*, **68**: 1123-1132.

Layton, R. L. (1985) Recreation, management and landscape in Epping Forest: c.1800-1984. *Field Studies,* **6**: 269-290.

Le Bohec, C., Gauthier-clerc, M., Grémillet, D., Pradel, R., Béchet, A., Gendner, J- P. and Le Maho, Y. (2007) Population dynamics in a long-lived seabird: I. Impact of breeding activity on survival and breeding probability in unbanded king penguins. *Journal of Animal Ecology*, **76** (6): 1149-1160.

Lebreton, J. -D., Burnham, K. P, Clobert, J. and Anderson, D. R. (1992) Modeling survival and testing biological hypotheses using marked animals: a unified approach with case studies. *Ecological Monographs*, **62** (1): 67-118.

Lecis, R. and Norris, K. (2003) Habitat correlates of distribution and local population decline of

the endemic Sardinian newt *Euproctus platycephalus*. *Biological Conservation*, **115**: 303-317.

LormÉe, H., Jouventin, P., Trouve, C. and Chastel, O. (2003) Sex-specific patterns in baseline corticosterone and body condition changes in breeding Red-footed Boobies *Sula sula*. *Ibis*, **145** (2): 212-219.

Levins, R. (1969) Some demographic and genetic consequences of environmental heterogeneity for biological control. *Bulletin of the Entomological Society of America*, **15** (3): 237-240.

Lips, K. R. (1998) Decline of a tropical mountain amphibian fauna. *Conservation Biology*, **12** (1): 106-117.

Lips, K. R., Burrowes, P. A., Mendelson III, J. R. and Parra-Olea, G. (2004) Amphibian declines in Latin America: widespread population declines, extinctions and impacts. *Biotropica*, **37** (2): 163-165.

Lips, K. R., Brem, F., Brenes, R., Reeve, J. D., Alford, R. A., Voyles, J., Carey, C., Livo, L., Pessier, A. P. and Collins, J. P. (2006) Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proceedings of the National Academy of Sciences of the United States of America*, **103** (9): 3165-3170.

Loman, J. and Andersson, G. (2007) Monitoring brown frogs *Rana arvalis* and *R. temporaria*, in 120 south Swedish ponds 1989-2005. Mixed trends in different habitats. *Biological Conservation*, **135**: 46-56.

Loman, J. and Madsen, T. (2010) Sex ratio of breeding common toads (*Bufo bufo*): influence of survival and skipped breeding. *Amphibia-Reptilia*, **31**: 509-524.

Lopez, J. E. and Pfister, C. A. (2001) Local population dynamics in metapopulation models: implications for conservation. *Conservation Biology*, **15** (6): 1700-1709.

Lowe, W. H. Likens, G. E. and Costenino, B. J. (2006) Self-organisation in streams: the relationship between movement behaviour and body condition in a head water salamander. *Freshwater Biology*, **51**: 5052-5062.

Loyau, A., Gomez, D., Moureab, B., Théry, M., Hart, N. S., Jalme, M. S., Bennett, A. T. D. and Sorci, G. (2007) Iridescent structurally based coloration of eyespots correlates with mating success in the peacock. *Behavioral Ecology*, **18** (6): 1123-1131.

McCallum, H. (2005) Inconclusiveness of chytridiomycosis as the agent in widespread frog declines. *Conservation Biology*, **19** (5): 1421-1430.

MacGregor, H. C. (1995) Crested Newts – ancient survivors. *British Wildlife*, **7** (1): 1-8.

MacKenzie, D. I., Bailey, L. L. and Nichols, J. D. (2004) Investigating species co-occurrence patterns when species are detected imperfectly. *Journal of Animal Ecology*, **73**: 546-555.

MacKenzie, D. I. and Kendall, W. L. (2002) How should detection probability be incorporated into estimates of relative abundance? *Ecology*, **82** (9): 2387-2993.

McLee, A. G. and Scaife, R. W. (1992) The colonisation by great crested newts (*Triturus cristatus*) of a water body following treatment with a piscicide to remove a large population of sticklebacks (*Gasterosteus aculeatus*). *British Herpetological Society Bulletin*, **42**: 6-10.

Malmgren, J. C. (2001) Allometry for sexual size dimorphism in newts of the genus *Triturus* (Caudata: Salamandridae). *Evolutionary Ecology of Newts*, PhD thesis, University of Örebro.

Malmgren, J. C. (2003) Fishy business! Sticklebacks and great crested newts. Extracts from the Herpetofauna Workers Meeting, 2003. *Great Crested News*, **Issue 5.**

Malmgren, J. C. (2007) Modelling terrestrial interactions and shelter use in great crested newts (*Triturus cristatus*). *Amphibia-Reptilia*, **28**: 205-215.

Malmgren, J. C. and Enghag, M. (2008) Female preference for male dorsal crests in great crested newts (*Triturus cristatus*). *Ethology and Evolution*, **20** (1): 71-80.

Malmgren, J. C. and Thollesson, M. (1999) Sexual size and shape dimorphism in two species of newts, *Triturus cristatus* and *T. vulgaris* (Caudata: Salamandridae). *Journal of Zoology, London*, **249** (2): 127-136.

Mandrillon, A. -L. and Saglio, P. (2007) Effects of embryonic exposure to conspecific chemical cues on hatching and larval traits in the common frog (*Rana temporaria*). *Chemoecology* **17**, 169-175.

Mandrillon, A -L. and Saglio, P. (2009) Effects of single and combined embryonic exposures to herbicide and conspecific chemical alarm cues on hatching and larval traits in the common frog (*Rana temporaria*). *Archives of Environmental Contamination Toxicology*, **56**: 566-576.

Manenti, R., Ficetola, G. F. and De Bernardi, F. (2009) Water, stream morphology and landscape: complex habitat determinants for the fire salamander *Salamandra salamandra*, *Amphibia-Reptilia*, **30**: 7-15.

Marco, A., Lizana, M., Alvarez, A. and Blaustein, A. R. (2001) Egg wrapping behaviour protects newt embryos from UV radiation. *Animal Behaviour,* **61**: 639-644.

Marnell, F. (1998) Discriminant analysis of the terrestrial and aquatic habitat determinants of the smooth newts (*Triturus vulgaris*) and the common frog (*Rana temporaria*) in Ireland. *Journal of Zoology, London*, **244**: 1-6.

Marsh, D. M. and Borrell, B. J. (2001) Flexible oviposition strategies in the túngara frogs and their implications for tadpole spatial distributions. *Oikos*, **93**: 101-109.

Marsh, D. M. and Goicochea, M. A. (2003) Monitoring terrestrial salamanders: biases caused by intense sampling and choice of cover objects. *Journal of Herpetology*, **37** (3): 460-466.

Marsh, D. M. and Trenham, P. C. (2001) Metapopulation dynamics and amphibian conservation. *Conservation Biology*, **15** (1): 40-49.

Marty, P., Angélibert, S., Giani, N. and Joly, P. (2005) Directionality of pre- and post- breeding migrations of a marbled newt population (*Triturus marmoratus*): implications for buffer zone management. *Marine and Freshwater Ecosystems*, **15**: 215-225.

Marvin, G. A. (2001) Age, growth and long-term site fidelity in the terrestrial Plethodontid salamander *Plethodon kentucki*. *Copeia*, **2001** (1): 108-117.

Mathis, A., Ferrari, M. C. O., Windel, N., Messier, F. and Chivers, D. P. (2008) Learning by embryos and the ghost of predation future. *Proceedings of the Royal Society, London B,* **275**: 2603-2607.

Matthews, K. R. and Miaud, C. (2007) A skeletochronological study of the age structure, growth, and longevity of the mountain yellow-legged frog, *Rana muscosa*, in the Sierra Nevada, California. *Copeia*, **2007** (4): 986-993.

May, A., Panter, J. and Williams, M. (2000) Management options for Epping Forest. *Geographical Review*, **14**: 34-39.

Mazerolle, M. J. (2001) Amphibian activity, movement patterns, and body size in fragmented peat bogs. *Journal of Herpetology*, **35** (1): 13-20.

Mazerolle, M. J. (2006) Improving data analysis in herpetology: using Akaike's Information Criterion (AIC) to assess the strength of biological hypotheses. *Amphibia-Reptilia*, **27**: 169-180.

Mazerolle, M. J., Bailey, L. L., Kendall, W. L., Royale, A., Converse, S. J. and Nichols, J. D. (2007) Making great leaps forward: accounting for detectability in herpetological field studies. *Journal of Herpetology*, **41** (4): 672-689.

Meredith, C. S. and Whiteman, H. H. (2008) Effects of nitrate on embryos of three amphibian species. *Bulletin of Environmental Contamination Toxicology*, **80**: 529-533.

Met Office (2011) http://www.metoffice.gov.uk/news/releases/archive/2011/cold-dec. Accessed 13 Dec 2011.

Miaud, C. (1993) Predation on newt eggs (*Triturus alpestris* and *T. helveticus*): identification of predators and protective role of oviposition behaviour. *Journal of Zoology, London,* **231**: 575-582.

Miaud, C. (1994) Role of wrapping behaviour in egg survival in three species of *Triturus* (Amphibia: Urodela). *Copeia*, **1994** (2): 535-537.

Miaud, C. (1995) Oviposition site selection in three species of European newts (Salamandridae) genus *Triturus*. *Amphibia-Reptilia*, **16**: 265-272.

Miaud, C., Andreone, F., Ribéron, A., De Michelis, S., Clima, V., Castanet, J., Francillon-Vieillot, H. and Guyétant, R. (2001) Variations in age, size at maturity and gestation duration among two neighbouring populations of the alpine salamander (*Salamandra lanzai*). *Journal of Zoology,* **254**: 251-260.

Miaud, C., Joly, P. and Castanet, J. (1993) Variation in age structures in a subdivided population of *Triturus cristatus*. *Canadian Journal of Zoology,* **71**: 1874-1879.

Miaud, C., Sanuy, D. and Avrillier, J. (2000) Terrestrial movements of the natterjack toad *Bufo calamita* (Amphibia, Anura) in a semi-arid, agricultural landscape. *Amphibia-Reptilia*, **21**: 357-369.

Miller, C. W. and Moore, A. J. (2007) A potential resolution to the lek paradox through indirect genetic effects. *Proceedings of the Royal Society: Biological Sciences*, **274** (1615): 1279-1286.

Mokany, A. and Shine, R. (2003) Oviposition site selection by mosquitoes is affected by cues from conspecific larvae and anuran tadpoles. Austral Ecology, **28** (1): 33-37.

Monello, R. J. and Wright, R. G. (1999) Amphibian habitat preferences among artificial ponds in the Palouse region of Northern Idaho. *Journal of Herpetology*, **33** (2): 298-303.

Moilanen, A. and Hanski, I. (1998) Metapopulation dynamics: effects of habitat quality and landscape structure. *Ecology,* **79** (7): 2503-2515.

Møller, A. P. and Alatalo, R. V. (1999) Good-genes effects in sexual selection. *Proceedings of the Royal Society, London B*, **266**: 85-91.

Monti, L., Hanter Jr, M. and Witham, J. (2000) An evaluation of the artificial cover object (ACO)

method for monitoring populations of the red backed salamander *Plethodon cinereus*. *Journal of Herpetology*, **34** (4): 624-629.

Montieth, K. E. and Paton, P. W. C. (2006) Emigration behaviour of spotted salamanders on golf courses in southern Rhode Island. *Journal of Herpetology*, **40** (2): 195-205.

Moya-Larani, J., Macias-Ordonez, R., Blankenhorn, W. U. and Fernandez-montraveta (2008) Analysing the body condition: mass, volume or density? *Journal of Animal Ecology*, **77**: 1099-1108.

Muths, E. (2003) Home range and movements of Boreal toads in undisturbed habitat. *Copeia*, **2003** (1): 160-165.

Muths, E., Scherer, R. D., Corn, P. S. and Lambert, B. A. (2006) Estimation of temporary emigration in male toads. *Ecology*, **87** (4): 1048-1056.

Muus, B. J. and Dahlstrøm, P. (1971) *The freshwater fishes of Britain and Europe*. Collins, London.

Najmanová, L. and Adamík, P. (2009) Effect of climatic change on the duration of the breeding season in three European thrushes. *Bird Study*, **56** (3): 349-356.

Norris, K. M and Hosie, C. A. (2005) A quantified ethogram for oviposition in *Triturus* newts: description and comparison for *T. helveticus* and *T. vulgaris*. *Ethology*, **111**: 357-366.

Nyström, P., Hansson, J., Månsson, J., Sundstedt, M., Reslow, C. and Broström, A. (2007) A documented amphibian decline over 40 years: possible causes and implications for species recovery. *Biological Conservation*, **138**: 399-411.

Oldham, R. S. and Swan, M. J. S. (1997). Pond loss and amphibians; historical perspective. In: J. Boothby Ed. *British Pond Landscapes: action for protection and enhancement.* Proceedings of the UK conference of the Pond Life Project, held at University College, Chester, 7th – 9th September, 1997.

Oldham, R. S., Keeble, J., Swan, M. J. S. and Jeffcote, M. (2000) Evaluating the suitability of habitat for the great crested newt (*Triturus cristatus*). *Herpetological Journal*, **10**: 143-155.

Olgun, K., Uzurn, N., Avci, A. and Miaud, C. (2005) Age, size and growth of the southern crested newt *Triturus karelinii* (Strauch, 1870) in a population from Bozdag (Western Turkey). *Amphibia-Reptilia*, **26**: 223-230.

Olivier, H. M. and Moon, B. R. (2010) The effects of atrazine on spotted salamander embryos and their symbiotic alga. *Ecotoxicology*, **19**, 654-661.

O'Steen, S., Eby, S. L. and Bunce, J. A. (2010) Dynamically honest displays: courtship locomotor performance indicates survival in guppies. *Functional Ecology*, **24** (5): 1045-1053.

Orizaola, G. and Braña, F. (2003) Oviposition behaviour and vulnerability of eggs to predation in four newt species (genus *Triturus*). *Herpetological Journal*, **13**: 121-124.

Orizaola, G. and Braña, F. (2006) Effect of salmonid introduction and other environmental characteristics on amphibian distribution and abundance in mountain lakes of northern Spain. *Animal Conservation*, **9**: 171-178

Ortiz, M. E., Marco, A., Saiz, N. and Lizana, M. (2004) Impact of ammonium nitrate on growth and survival of six European amphibians. *Archive of Environmental Contamination and Toxicology*, **47**: 234-239.

Ouellet, M., Mikaelian, I., Pauli, B. D., Rodrigue, J. and Green, D. M. (2003) Historical evidence of widespread chytrid infection in North American amphibian populations. *Conservation Biology*, **19** (5): 1431-1440.

Ozgul, A., Armitage, K. B., Blumstein, D. T., VanVuren, D. H. and Oli, M. K. (2006) Effects of patch quality and network structure on patch occupancy dynamics of a yellow-bellied marmot metapopulation. *Journal of Animal Ecology*, **75** (1): 191-202.

Panhuis, T. M., Butlin, R., Zuk, M. and Tregenza, T. (2001) Sexual selection and speciation. *Trends in Ecology and Evolution*, **7** (16): 354-371.

Parris, K. M. (2006) Urban amphibian assemblages as meta communities. *Journal of Animal Ecology*, **75** (3): 757-764.

Pechmann, J. H. K., Scott, D. E., Semlitsch, R. D., Caldwell, J. P., Vitt, L. J. and Gibbons, J. W. (1991) Declining amphibian populations: the problem of separating human impacts from natural fluctuations. *Science*, (New Series), **253** (5022): 892-895.

Pechmann, J. H. K. and Wilbur, H. M. (1994) Putting declining amphibian populations into perspective: natural fluctuations and human impacts. *Herpetologica*, **50** (1): 65-84.

Peig, J. and Green, A. J. (2010) The paradigm of body condition: a critical reappraisal of current methods based on mass and length. *Functional Ecology*, **24**: 1323-1332.

Pellet, J., Helfer, V. and Yannic, G. (2007) Estimating population size in European tree frog (*Hyla arborea*) using individual recognition and chorus counts. *Amphibia-Reptilia*, **28**: 287-294.

Pellet, J., Schmidt, B. R., Fivaz, F., Perrin, N. and Grossenbacher, K. (2006) Density, climate

and varying return points: an analysis of long-term population fluctuations in the threatened European tree frog. *Oecologia*, **149**: 65-71.

Penna, M. and Velásquez, N. (2010) Heterospecific vocal interactions in a frog from the southern temperate forest, *Batrachyla taeniata*. *Ethology*, **117** (1): 63-71.

Peter, A -K, H. (2001) Dispersal rates and distances in adult water frogs, *Rana lessonae*, *R. ridibunda*, and their hybridogenetic associate *R. esculenta*. *Herpetologica*, **57** (4): 449-460.

Petranka, J. W. and Holbrook, C. T. (2006) Wetland restoration for amphibians: should local sites be designed to support metapopulations or patchy populations? *Restoration Ecology*, **14** (3): 404-411.

Pickett, J. (1986) Notes on the reptiles and amphibians of Epping Forest, past, present and future. *British Herpetological Society Bulletin*, **17**: 37-44.

Pledger, S., Pollock, K. H. and Norris, J. L. (2003) Open capture-recapture models with heterogeneity: I. Cormack-Jolly-Seber model. *Biometrics*, **59** (786-794).

Pollock, K. H. (1982) A capture–recapture design robust to unequal probability of capture. *Journal of Wildlife Management*, **46**: 757–760.

Pounds, A. J, Bustamante, M. R., Coloma, L. A., Consuegra, J. A., Fogden, M. P. L., Foster, P. N., La Marca, E., Masters, K. L., Merino-Viteri, A., Puschendorf, R., Ron, S. R., Sánchez-Azofeifa, G. A., Still, C. J. and Young, B. E. (2006) Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature*, **439** (12th January) 161-167.

Pulliam, H. R. (1988) Sources, sinks and population regulation. *The American Naturalist*, **132** (5): 652-661.

Purrenhage, J. L., Niewiarowski, P. H. and Moore, F. B -G (2009) Population structure of spotted salamanders (*Ambystoma maculatum*) in a fragmented landscape. *Molecular Ecology*, **18**: 235-247.

Rachowicz, L. J., Hero, J -M., Alford, R. A., Taylor, J. W., Morgan, J. A. T., Vredenburg, V. T., Collins, J. P. and Briggs, C. J. (2005) The novel and endemic pathogen hypothesis: competing explanations for the origin of emerging infectious diseases of wildlife. *Conservation Biology*, **19** (5): 1441-1448.

Raymond, L. R. and Hardy, L. M. (1990) Demography of a population of *Ambystoma talpoideum* (Caudata: Ambystomidae) in Northwestern Louisiana. *Herpetologica*, **46** (4): 371-382.

Reading, C. J. (1998) The effect of winter temperatures on the timing of breeding activity in the common toad *Bufo bufo*. *Oecologia*, **117** (4): 469-475.

Reading, C. J. (2007) Linking global warming to amphibian declines through its effects on female body condition and survivorship. *Oecologia*, **151**: 125-131.

Reading, C. J. (2010) The impact of environmental temperature on larval development and metamorph body condition in the common toad, *Bufo bufo*. *Amphibia-Reptilia*, **31**: 483-488.

Reed, D. H., O'Grady, J. J., Brook, B. W. Ballou, J. D. and Frankham, R. (2003) Estimates of minimum viable population sizes for vertebrates and factors influencing those estimates. *Biological Conservation*, **113**: 23-34.

Regosin, J. V., Windmiller, B. S. and Reed, J. M. (2003) Terrestrial habitat use and winter densities of the wood frog (*Rana sylvatica*). *Journal of Herpetology*, **37** (2): 390-394.

Relyea, R. A. (2001) The relationship between predator risk and antipredator responses in larval anurans. *Ecology*, **82** (2): 541-554.

Relyea, R. A. (2002a) The many faces of predation: How induction, selection and thinning combine to alter prey phenotypes. *Ecology,* **83** (7): 1953-1964.

Relyea, R. A. (2002b) Costs of phenotypic plasticity. *The American Naturalist*, **159** (3): 272-282.

Relyea, R. A. (2003) How prey respond to combined predators: a review and an experimental test. *Ecology,* **84**: 1827-1839.

Relyea, R. A. (2004) Fine tuned phenotypes: tadpole plasticity under 16 combinations of predators and competitors. *Ecology*, **85** (1): 172-179.

Relyea, R. A. and Mills, N. (2001) Predator-induced stress makes the pesticide carbaryl more deadly to gray treefrog tadpoles (*Hyla versicolor*). *Proceedings of the National Academy of Sciences of the United States of America*, **98** (5): 2491-2496.

Relyea, R. A. and Werner, E. E. (2000) Morphological plasticity in four larval anurans distributed along an environmental gradient. *Copeia*, **2000** (1): 178-190.

Resetarits, W. J. (1996) Oviposition site choice and life history evolution. *American Journal of Zoology*, **36** (2): 205-215.

Richardson, C., Lena, J -P. and Lengagne, T. (2008) Are leaders good mates? A study of call timing and male quality in a chorus situation. *Animal Behaviour*, **76** (5): 1487-1485.

Richter, S. C. and Seigel, R. A. (2002) Annual variation in the population ecology of the endangered gopher frog, *Rana sevosa* Goin and Netting. *Copeia*, **2002** (4): 962-972.

Ries, C., Spaethe, J., Sztatecsny, M., Strondl, C. and Hödl, W. (2008) Turning blue and ultraviolet: sex-specific colour change during the mating season in the Balkan moor frog. *Journal of Zoology*, **276** (3): 229-236.

Roberts, J. M. and Griffiths, R. A. (1992) The dorsal stripe in newt efts: a method for distinguishing *Triturus vulgaris* from *T. helveticus*. *Amphibia-Reptilia*, **13** (1): 13-19.

Rödel, J. R., Bora, A., Kaetzke, P., Khaschei, M., Hutzelmeyer, H. and von Holst, D. (2004) Over-winter survival in subadult European rabbits: weather effects, density dependence, and the impact of individual characteristics. *Oecologia*, **140** (4): 566-576.

Romansic, J. M., Higashi, E. M., Diez, K. A. and Blaustein, A. R. (2007) Susceptibility of newly-metamorphosed frogs to the pathogenic water mould (*Saprolegnia* sp.) *The Herpetological Journal*, **17** (3): 161-166.

Saenz, D., Johnson, J. B., Adams, C. K. and Jaylon, G. H. (2003) Accelerated hatching of Southern Leopard Frog (*Rana sphenocephala*) eggs in response to the presence of a crayfish (*Procambarus nigrocinctus*) predator, *Copeia*, **2003**: 646-649.

Salvidio, S. (2009) Detecting amphibian population cycles: The importance of appropriate statistical analyses. *Biological Conservation*, **142**: 455-461.

Sandercock, B. K. (2006) Estimation of demographic parameters from live-encounter data: a summary review. *The Journal of Wildlife Management*, **70** (6): 1504-1520.

Schabetsberger, R., Jehle, R., Maletzky, A., Pesta, J. and Sztatecsay, M. (2004) Delineation of terrestrial reserves for amphibians: post breeding migrations of Italian crested newts (*Triturus c. carnifex*) at high altitude. *Biological Conservation*, **117** (2004): 95-104.

Schaub, M., Gimenez, O., Schmidt, B. R. and Pradel, R. (2004) Estimating survival and temporary emigration in the multistate capture-recapture framework. *Ecology*, **85** (8): 2107-2113.

Scherer, R. D., Muths, E., Noon, B. R. and Corn, P. S. (2005) An evaluation of weather and disease as causes of decline in two populations of boreal toads. *Ecological Applications*, **15** (6): 2150-2160.

Schmidt, B. R. (2003) Count data, detection probabilities and the demography, dynamics, distribution, and decline of amphibians. *Comptes Rendus Biologies*, **326** (S1): 119-124.

Schmidt, B. R. (2005) Monitoring the distribution of pond-breeding amphibians when species are detected imperfectly. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **15**: 681-692.

Schmidt, B. R. and Anholt, B. R. (1999) Analysis of survival probabilities of female common toads, *Bufo bufo*. *Amphibia-Reptilia*, **20**:97-108.

Schmidt, B. R., Feldmann, R. and Schaub, M. (2004) Demographic processes underlying population growth and decline in *Salamandra salamandra*. *Conservation Biology*, **19** (4): 1149-1156.

Schmidt, B. R., Schaub, M. and Anholt, B. R. (2002) Why you should use capture-recapture methods when estimating survival and breeding probabilities: on bias, temporary emigration, overdispersion and common toads. *Amphibia-Reptilia*, **23**: 375-388.

Schmidt, B. R., Schaub, M. and Steinfartz, S. (2007) Apparent survival of the salamander *Salamandra salamandra* is low because of high migratory activity. *Frontiers in Zoology*, **4**: 19-25.

Schulte-Hostedde A. I., Millar J. S., Hickling G. J. (2001) Evaluating body condition in small mammals. *Canadian Journal of Zoology*, **79** (6): 1021-1029.

Seale, D. B. (1982) Physical factors influencing oviposition by the wood frog, *Rana sylvatica*, in Pennsylvania. *Copeia*, **1982** (3): 627-635.

Semlitsch, R. D. (1988) Allotopic distribution of two salamanders: effects of fish predation and competitive interactions. *Copeia*, **1988** (2): 290-298.

Semlitsch, R. D. (2000) Principles for management of aquatic breeding amphibians. *The Journal of Wildlife Management*, **64** (3): 615-631.

Semlitsch, R. D, Scott, D. E., Pechmann, J. H. K. and Gibbons, J. W. (1993) Phenotypic variation in the arrival time of breeding salamanders: individual repeatability and environmental influences. *Journal of Animal Ecology*, **62** (2): 334-340.

Serrano-Meneses, M. A., Córdoba-Aguilae, A., Méndez, V., Layen, S. J. and Székely, T. (2006) Sexual size dimorphism in the American rubyspot: male body size predicts male competition and mating success. *Animal Behaviour*, **73** (6): 987-997.

Sever, D. M., Halliday, T., Moriarty. E. C. and Araño, B. (2001) Sperm storage in females of the smooth newt (*Triturus v. vulgaris* L.): II. Ultrastructure of the spermathecae after the breeding season. *Acta Zoologica*, **82**: 49-56.

Seymour, R. S. and Bradford, D. F. (1995) Respiration of amphibian eggs. *Physiological Zoology*, **68** (1): 1-25.

Sexton, O. J., Philips, C. and Bramble, J. E. (1990) The effects of temperature and precipitation

on the breeding migration of the spotted salamander (*Ambystoma maculatum*). *Copeia*, **1990** (3): 781-787.

Shaffer, M. L. (1981) Minimum population sizes for species conservation. *Bioscience*, **31** (2): 131-134.

Sih, A., Kats, L. B. and Moore, R. D. (1992) Effect of predatory sunfish on the density, drift and refuge use of stream salamander larvae. *Ecology*, **73** (4):1418-1430.

Sih, A. and Maurer, E. (1992) Effects of cryptic oviposition on egg survival for stream-breeding streamside salamanders. *Journal of Herpetology*, **26** (1): 114-116.

Sinsch, U. (1997) Post-metamorphic dispersal and recruitment of first breeders in a *Bufo calamita* metapopulation. *Oecologia*, **112**: 42-47.

Sinsch, U. (1988) Seasonal changes in the migratory behaviour of the toad *Bufo bufo*: direction and magnitude of movement. *Oecologia*, **76** (3): 390-398.

Sjogren-Gulve, P. (1994) Distribution and extinction patterns within a northern metapopulation of the pool frog, *Rana lessonae*. *Ecology*, **75** (5): 1357-1367.

Skei, J. K., Dolmen, D., Rønning, L. and Ringsby, T. H. (2006) Habitat use during the aquatic phase of the newts *Triturus vulgaris* (L.) and *T. cristatus* (Laurenti) in central Norway: proposition for a conservation and monitoring area. *Amphibia-Reptilia*, **27**: 309-324.

Slos, S., De Meester, L. and Stoks, R. (2009) Food level and sex shape predator-induced physiological stress: immune defence and antioxidant defence. *Oecologia*, **161**: 461-467.

Slos, S. and Stoks, R. (2008) Predation risk induces stress proteins and reduces antioxidant defence. *Functional Ecology*, **22**: 637-642.

Ślusarczyk, M. (1999) Predator-induced diapause in *Daphnia magna* may require two chemical cues. *Oecologia*, **119**: 159-165.

Smith, G. R., Boyd, A., Dayer, C. B., Ogle, M. E. and Terlecky, A. J. (2009) Responses of grey treefrog and American toad tadpoles to the presence of cues from multiple predators. *Herpetological Journal*, **19** (2): 79-83.

Smith, C. K. and Petranka, J. W. (2000) Monitoring terrestrial salamanders: repeatability and validity of area-constrained cover object searches. *Journal of Herpetology*, **34** (4): 547-557.

Smith, M. A. and Green, D. M. (2005) Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? *Ecography*,

28 (1): 110-128.

Spence, R., Ashton, R. and Smith, C. (2007) Oviposition decisions are mediated by spawning site quality in wild and domesticated zebrafish, *Danio rerio*. *Behaviour*, **144**: 953-966.

Stav, G., Kotler, B. P. and Blaustein, L. (2007) Direct and indirect effects of dragonfly (*Anax imperator*) nymphs on green toad (*Bufo viridis*) tadpoles. *Hydrobiologia*, **579**: 85-93.

Stevens, V. M. and Baguette, M. (2008) Importance of habitat quality and landscape connectivity for the persistence of endangered natterjack toads. *Conservation Biology*, **22** (5): 1194-1204.

Storfer, A. (2003) Amphibian declines: future directions. *Diversity and Distributions*, **9**: 151-163.

Stuart, S. N., Chanson, J. S., Cox, N. A., Young, B. E., Rodrigues, A. S. L., Fischman, D. L. and Waller, R. W. (2004) Status and trends of amphibian declines and extinctions worldwide. *Science*, **306** (5702): 1783-1786.

Sullivan, B. K. and Fernandez, P. J. (1999) Breeding activity, estimated age-structure, and growth in Sonoran Desert anurans. *Herpetologica*, **55** (3): 334-343.

Sullivan, B. K., Ryan, M. J. and Verrell, P. A. (1995) Female choice and the mating system structure. In: *Amphibian Biology, vol. 2.* Eds H. Heatwole and B. K. Sullivan, Surrey Beatty and Sons, Chipping Norton, Australia.

Swan, M. J. S. and Oldham, C. S. (1993) *Herptile sites vol. 1: National amphibian survey. Final report. No. 38 – English Nature research reports*. English Nature, Peterborough.

Sztatecsny, M. and Schabetsberger, R. (2005) Into thin air: vertical migration, body condition and quality of terrestrial habitats of alpine common toads, *Bufo bufo*. *Canadian Journal of Zoology*. **83**: 788-796.

Teacher, A. G. F., Cunningham, A. A. and Garner, T. W. J. (2010) Assessing the long-term impact of *Ranavirus* infection in wild common frogs. *Animal Conservation*, **2010**: 1-9.

Temple, H. J. and Cox, N. A. (2009) *European Red List of Amphibians*. Luxembourg: Office for official Publications of the European Communities.

Teplitsky, C. Plénet, S. and Joly, P. (2003) Tadpoles' response to risk of fish introduction. *Oecologica*, **134** (2): 270-277.

The Conservation of Habitats and Species Regulations (2010) http://www.legislation.gov.uk/uksi/2010/490/made. Accessed Nov. 2011.

Timm, B. C., McGarigal, K. and Gamble, L. R. (2007) Emigration timing of juvenile pond-breeding amphibians in Western Massachusetts. *Journal of Herpetology*, **41** (2): 243-

250.

Tonn, W. M., Holopainen, I. J. and Paszkowski, C. A. (1994) Density-dependent effects and the regulation of crucian carp populations in single-species ponds. *Ecology*, **75** (3): 824-834.

Tóth, Z., Hoi, H. and Hettyey, A. (2011) Kin discrimination during egg-cannibalism in smooth newts: does competition matter? *Journal of Zoology*, **284**: 46-52.

Trivers, R. L. (1972) Parental investment and sexual selection. Pages 1871-1971. *In.* Campbell ed. *Parental investment and the descent of man.* Aldine, Chicago.

Van Buskirk, J. (2002) Phenotypic lability and the evolution of predator-induced plasticity in tadpoles. *Evolution*, **56**: 361-370.

Van Buskirk, J. and Arioli, M. (2002) Dosage response of an induced defence: how sensitive are tadpoles to predation risk? *Ecology*, **83** (6): 1580-1585.

Van Buskirk, J., McCollum, S. A. and Werner, E. E. (1997) Natural selection for environmentally induced phenotypes in tadpoles. *Evolution*, **51** (6): 1983-1992.

Van Buskirk, J. and McCollum, S. A. (2000) Functional mechanisms of an inducible defence in tadpoles: morphology and behaviour influence mortality risk from predation. *Journal of Evolutionary Biology*, **13**: 336-347.

Van Buskirk, J. and Schmidt, B. R. (2000) Predator-induced phenotypic plasticity in larval newts: trade-offs, selection and variation in nature. *Ecology*, **81** (11): 3009-3028.

Vanpé, C., Gaillard, J -M., Kjellander, P., Mysterud, A., Magnien, P., Delorme, D., Van Laere, G., Klein, F., Liberg, O. and Mark Hewison, A. J. (2007) Antler size provides an honest signal of male phenotypic quality in roe deer. *The American Naturalist*, **169** (4): 481-493.

Verrell, P. A. (1985) The winter habitat of amphibians in Milton Keynes, England. *British Herpetological Society Bulletin,* **13**: 24-77.

Verrell, P. A. (1987) The directionality of migrations of amphibians to and from a pond in southern England, with particular reference to the smooth newt, *Triturus vulgaris*. *Amphibia-Reptilia*, **8**: 93-100.

Verrell, P. and Halliday, T. (1985) The population dynamics of the crested newt *Triturus cristatus* at a pond in southern England. *Holarctic Ecology*, **8**: 151-156.

Vos, C. C., Goedhurt, R. W., Lammertsma, D. R., Spitzen-van sluijs, A. M. (2007) Matrix permeability of agricultural landscapes: an analysis of movements of the common frog

(*Rana temporaria*). *Herpetological Journal*, **17** (3): 174-182.

Warkentin, K. M., Currie, C. R. and Rehner, S. A. (2001) Egg-killing fungus induces early hatching of red-eyed tree frogs. *Ecology*, **82** (10): 2860-2869.

Watson, W. (1994) Construction of hibernacula for great crested newts. In: *Conservation and management of great crested newts: proceedings of a symposium held on 11th January 1994 at Kew gardens, Richmond, Surrey*. Eds T. Gent and R. Bray. English Nature science report No. 20. English Nature, Peterborough.

Weber, A. (2003) More than one 'fish kairomone'? Perch and stickleback kairomones affect *Daphnia* life history traits differently. *Hydrobiologia*, **498**: 143-150.

Weidong, G. and Swihart, R. K. (2004) Absent or present? Effects of non-detection of species occurrence on wildlife habitat models. *Biological Conservation*, **116**: 195-203.

Weladji, R. B. and Holand, Ø. (2003) Global climate change and reindeer: effects of winter weather on the autumn weight and growth of calves. *Oecologia*, **136** (2): 317-323.

Werner, E. E., Yurewicz, K. L., Skelly, D. K. and Relyea, R. A. (2007) Turnover in an amphibian metacommunity: the role of local and regional factors. *Oikos*, **116**: 1713-1725.

Wheeler, A. (1992) Fishes. In: *Epping Forest through the Eye of the Naturalist. Essex Naturalist No. 11*. Ed M. W. Hanson. The Essex Field Club, London.

Wheeler, B. A., Prosea, E., Mathis, A. and Wilkinson, R. F. (2003) Population declines of a long-lived salamander: a 20+ -year study of hellbenders, *Cryptobranchus alleganiensis*. *Biological Conservation*, **109**: 151-156.

White, G. C., and Burnham, K. P. (1999) Program MARK: survival estimation from populations of marked animals. *Bird Study*, **46** (1: supplement): S120-S139.

White, G. C. and Cooch, E. Eds. (2012) *Program MARK: a gentle introduction, 11th edition*, http://www.phidot.org/software/mark/docs/book/

Williams, C. (1999) *Metapopulation of the crested newt, Triturus cristatus*. PhD Thesis, Durrell Institute of Conservation and Ecology, University of Kent.

Willson, J. D., Winne, C. T. and Todd, B. D. (2011) Ecological and methodological factors affecting detectability and population estimation in elusive species. *Journal of Wildlife Management*, **75** (1): 36-45.

Wood, P. J., Greenwood, M. T. and Agnew, M. D. (2003) Pond biodiversity and habitat loss in the UK. *Area*, **35** (2): 206-216.

Wood, K. V., Nichols, J. D., Percival, H. F., and Hines, J. E. (1998) Size-sex variation in survival

rates and abundance of pig frogs, *Rana grylio*, in northern Florida wetlands. *Journal of Herpetology*, **32**(4): 527-535.

Wood, P. J., Greenwood, M. T. and Agnew, M. D. (2003) Pond biodiversity and habitat loss in the UK. *Area*, **35** (2): 206-216.

Wood, S. E. and Willoughby, L. G. (1986) Ecological observations on the fungal colonization of fish by Saprolegniacaea in Windermere. *The Journal of Applied Ecology*, **23** (3): 737-749.

Wright, L. J., Hoblyn, R. A., Green, R. E., Bowden. C. G. R., Mallord, J. W., Sutherland, W. J. and Dolman, P. M. (2009) Importance of climatic and environmental change in the demography of a multi-brooded passerine, the woodlark *Lullula arborea*. *Journal of Animal Ecology*, **78** (6): 1191-1202.

Yalden, D. W. (1967) Reptiles and amphibians in the London area, 1965-1966. *The London Naturalist*, **46**: 68-75.

Yoshino, K., Koga, T. and Oki, S. (2011) Chelipeds are the real weapon: cheliped size is a more effective determinant than body size in male-male competition for mates in a hermit crab. *Behavioural Ecology and Sociobiology*, **65** (9): 1825-1832.

Zahavi, A. (1975) Mate selection – a selection for a handicap. *Journal of Theoretical Biology*, **53**: 205-214.

Zeh, J., Poot, D., Miller, G., Koski, W., Baraff, L. and Rugh, D. (2002) Survival of bowhead whales *Balaena mysticetus* estimated from 1981-1998 photo identification data. *Biometrics*, **58** (4): 832-840.